EDGE COVID-19 documentation

Los Alamos National Laboratory

Email us: edge-covid19@lanl.gov
v1.0.16

Overview

EDGE COVID-19 *(EC-19)* is a tailored bioinformatics platform based on the more flexible and fully open-source EDGE Bioinformatics software (Li et al. 2017). This mini-version consists of a user-friendly GUI that drives standardized workflows for genome reference-based 'assembly' and preliminary analysis of Illumina or Oxford Nanopore (ONT) or PacBio data for SARS-CoV-2 genome sequencing projects. **The result is a final SARS-CoV-2 genome ready for submission to GISAID and GenBank**.

The default workflow in *EDGE COVID-19* includes (**Figure 1/Table 1)**:
1) data quality control (QC) and filtering,
2) alignment of reads to the original (first available) reference genome (NC_045512.2, we removed the PolyA tail from the 3' end (33 nt)),
3) creation of a consensus genome sequence based on the read alignments, and
4) variant analyses, with details on location (For eg. coding region. Synonymous changes, etc.)

The *EDGE COVID-19* platform can process Illumina, ONT data and PacBio, including ONT data from the SARS-CoV-2 ARTIC network sequencing protocols. Users can input/upload Illumina or ONT or PacBio FASTQ files (and/or download from NCBI SRA). For Illumina data, default analyses include read QC, read mapping to the reference, and variant analysis. For PacBio data, the read doesn't perform QC. For ONT data, the data must be demultiplexed prior to uploading; the samples will be processed individually. Also, the variant calling is not on by default for ONT. However, other functions (e.g. *de novo* assembly for whole genome data) are also available for these sequencing platforms. While command line execution is possible (see here and here), the GUI provides an easy data submission and results viewing platform, with the graphical and tabular views of variant/SNP data and a genome browser to view read coverage and location of SNPs or variants, as well as the reference annotations.

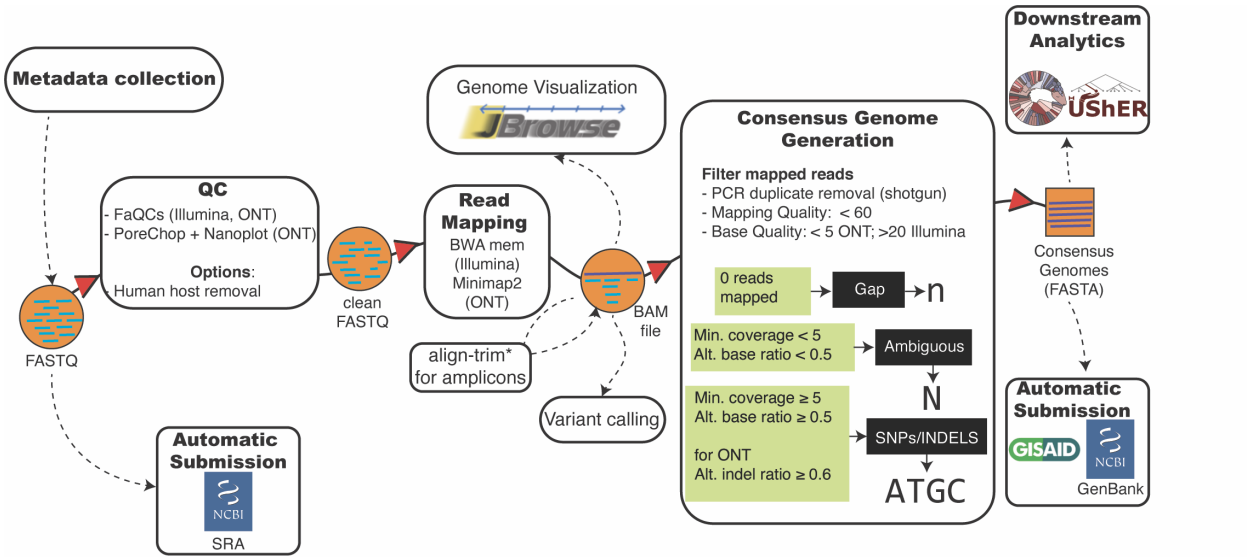


Figure 1: Overview of EDGE COVID-19 workflow. The workflow includes Quality Control (QC) and removal of low quality data in raw reads (FaQCs v2.09 (Lo and Chain, 2014), Porechop v0.2.3 (Wick, et al., 2017), mapping reads to a SARS-CoV-2 reference genome sequence using BWA v0.7.12 (Li and Durbin, 2009) (default for Illumina) or minimap2 v2.17(Li, 2018) (default for ONT), removing primer sequences (modified ver. of *align_trim*) for genomes sequenced using amplicon-based enrichment strategies (like ARTIC (Tyson, et al., 2020) , SWIFT, CDC-SC2 (Paden, et al., 2020), etc.), generation of consensus genomes, variant calling (using SAMtools v1.10 and BCFtools v1.10.2 (Li, 2011), lineage call using Pangolin v3.1.16, and phylogenetic placement using UsheR v0.5.1. Dotted line indicates optional steps.

Table 1: EDGE COVID-19 Workflows summary

	EC19-ONT	EC19-ILLUMINA	EC19-PacBio
QC	FaQCs -q 7 -min_L 350	FaQCs -q 20 -min_L 50	NA
Primer trimming	*align_trim**	*align_trim**	*align_trim**
Mapping/Aligner	minimap2	BWA	minimap2
Mapping/Aligner args	--MD -La	mem	--MD -La -x map-pb
Filter clipped alignment length (SamClip[$])	150	50	150
Variant call/Consensus genomes tool	samtools mpileup	samtools mpileup	samtools mpileup
Variant call parameters	-q 60 -Q 5 -d 0 -A -B -a	-q 60 -Q 20 -d 0 -A -B -a	-q 60 -Q 5 -d 0 -A -B -a
Consensus[&] parameters	--propThresh=0.5 --covThresh=5 --baseQual=5 --hetpropThresh=0.2 --filterHomopolymer --filterStrandBias	--propThresh=0.5 --covThresh=5 --baseQual=20 --hetpropThresh=0.2	--propThresh=0.5 --covThresh=5 --baseQual=5 --hetpropThresh=0.2 --filterHomopolymer
Consensus parameters Indel specific	--INDELpropThresh=0.6	--INDELpropThresh=0.5	--INDELpropThresh=0.6

$ SamClip https://github.com/tseemann/samclip

* align_trim https://github.com/LANL-Bioinformatics/EDGE/blob/SARS-CoV2/scripts/align_trim.py

& consensus workflow https://gitlab.com/chienchi/reference-based_assembly

for non-amplicon methods (no align_trim), PCR deduplication is also performed by `samtools markdup -r -s`

We have tested these workflows using Illumina (e.g. SRR11393704) and ONT (e.g. SRR11397722) datasets; these projects (along with a few others) are made public on the site. The workflow is also available as a Docker container (https://hub.docker.com/r/bioedge/edge-covid19), able to run on any local hardware infrastructure.

Note: For EDGE Bioinformatics users who would also like to use the phylogeny or read- and assembly-based taxonomy classification tools to identify all organisms that may be present within complex samples, we recommend using the original EDGE Bioinformatics platform which harbors several tools and associated (large) databases that enable such a search. *In initial tests of taxonomy classification of SARS-CoV-2 samples (with no SARS-CoV-2 genomes in any of the databases), we recover SARS coronavirus and Bat coronavirus as the nearest neighbors (See table below).*

Columns...

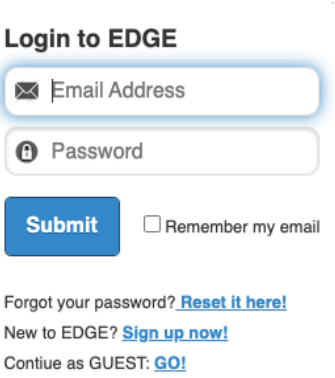
Tool	#Reads	%Reads	Level	Top1	Top2	Top3	Top4	Top5
gottcha-strDB-v	7,827	6.0	strain	SARS coronavirus	Bat coronavirus BM48-31/BGR/2008	N/A	N/A	N/A
pangia	5,008	3.9	strain	SARS coronavirus	Bat coronavirus BM48-31/BGR/2008 strain BtCoV/BM48-31/BGR/2008	N/A	N/A	N/A
metaphlan2	0	0.0	strain	N/A	N/A	N/A	N/A	N/A
bwa	3,296	2.5	strain	Bat coronavirus Rp/Shaanxi2011	Bat coronavirus Cp/Yunnan2011	BtRs-BetaCoV/HuB2013	Rhinolophus affinis coronavirus	Bat SARS-like coronavirus RsSHC014
kraken2	48,317	37.2	strain	SARS coronavirus	Rhodococcus opacus PD630	Burkholderia dolosa PC543	Xanthomonas citri pv. fuscans	Aeromonas hydrophila ML09-119

A step by step guide for running *EC-19*:

Visit https://edge-covid19.edgebioinformatics.org/ and follow the steps below:

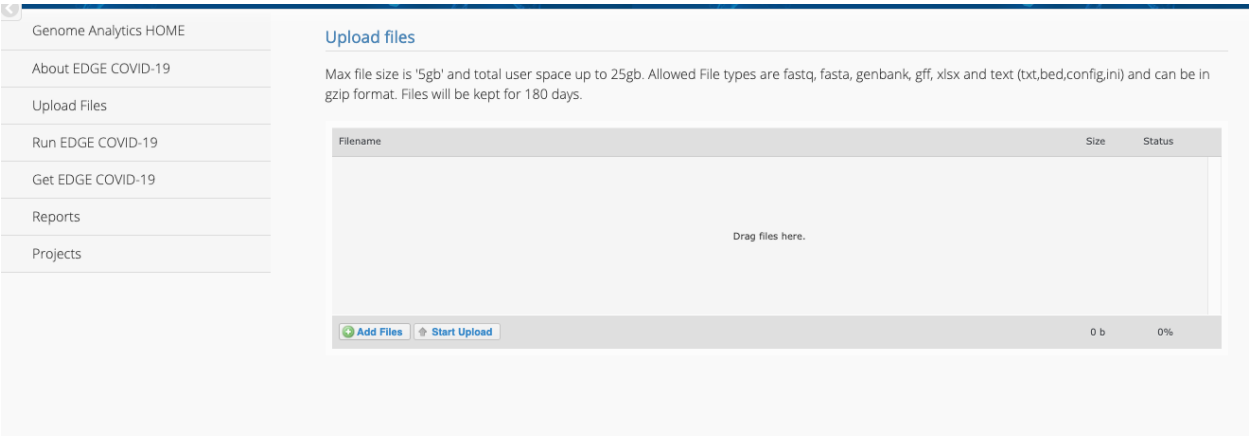
Step 1: Create an account

You need to create an account. Click the "Sign up" link in the upper right corner of the page. After you have an account, you can click "Log in" for all subsequent visits and provide your user information. If you don't want to create an account, the "GUEST" account is provided for anonymous login with data constraint to keep 24 hours only.



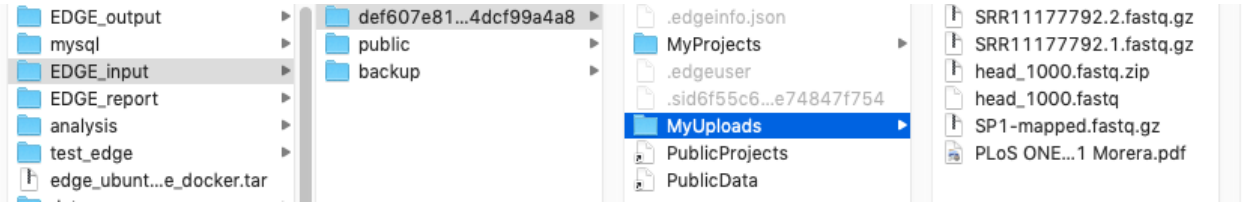
Step 2: Upload your raw reads

After you have logged in, you can click on “Upload Files” in the left menu. Drag and drop your data files into the window provided. Click “Start Upload” when you have added the files you need. The files will be put in a folder called MyUploads. The maximum file size is 5 gb and the total user upload space is 25 gb. The data will be stored for 180 days (It will be adjusted depends on the system resource and users will be notified) and user can clean up their uploaded data in the user icon popup menu.

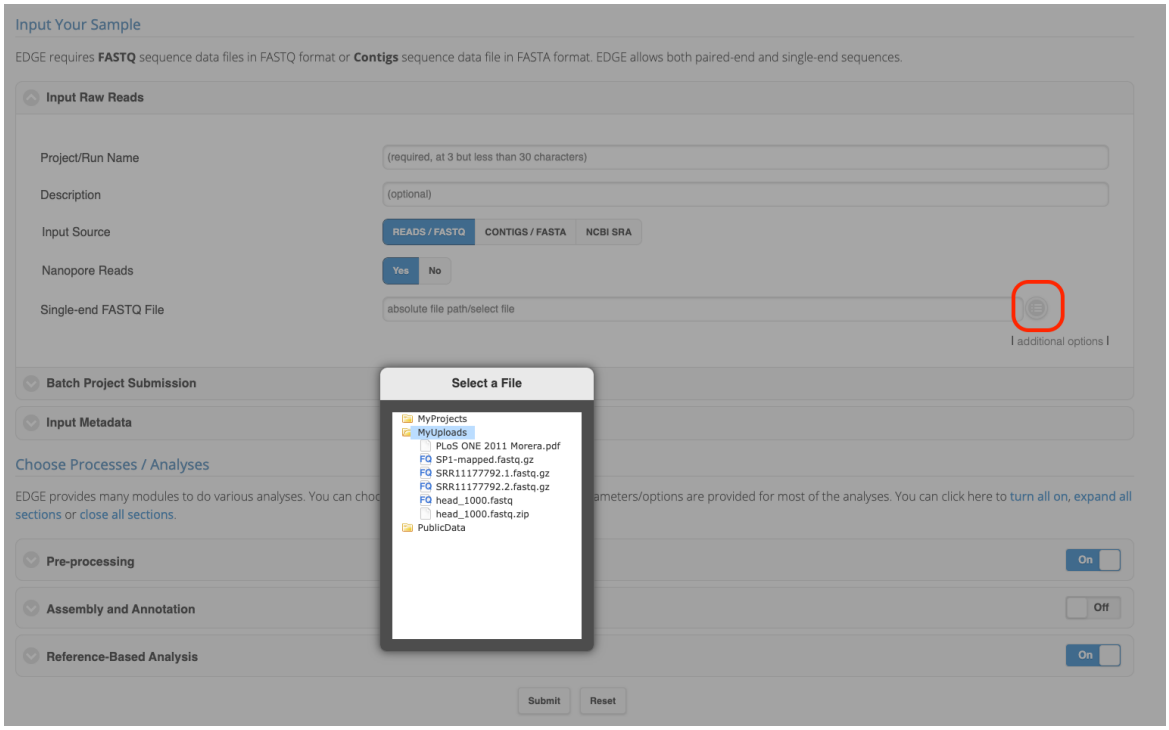


For a local installation:

The easiest way to upload your data is to put your data files in the upload folder, which can be found within the `EDGE_input` folder that you created when installing *EDGE COVID-19* docker [see here] . Within `EDGE_input`, there will be a folder with a long string of characters as the name and within that folder, there will be a folder called `MyUploads` where you can put your raw reads. This folder can then be seen from the web server (http://localhost/) by clicking on the button next to boxes where you input your FASTQ files.



The `MyUploads` folder can be seen from the web server by clicking on the button to the right of the box(es) where you input your FASTQ files. (See figure below.) Click the file(s) you want to analyze.



Step 3: Run your sample

If you are familiar with the EDGE Bioinformatics environment then you can skip this step and jump right into analyzing your data. Even if you are not familiar, you may still be able to skip this step, as EDGE Bioinformatics has a relatively intuitive design to instinctively get to your analyses right away. However, for completeness, here is a short description that will get you started. For a more detailed description, you can also visit our documentation site for EDGE Bioinformatics at https://edge.readthedocs.io.

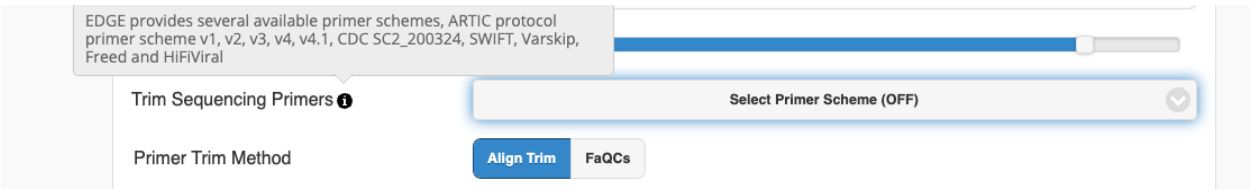
In the *EDGE COVID-19* web server,

1. Type in a unique **Project/Run Name** with no spaces, but use underscores and/or dases, if needed.
2. Write in a short **Description.** Spaces are allowed.

3. In the **Input Source** section, select **READS/FASTQ** for analyzing your own raw reads or **NCBI SRA** if you want to analyze COVID-19 samples deposited in SRA.
4. Select **Platform** with **Nanopore** if your sample was generated using Nanopore; select **illumina** or **PacBio** if sample was from the corresponding platform.
5. Input your raw reads by clicking on the button to the right of the input box (highlighted with a red box in the figure above) and then within the GUI navigate to your **MyUploads** folder where you have added your raw reads in Step 2.
6. You can skip the `**Batch Project Submission**`, if you are only processing one sample. A detailed instructions on using the batch mode can be found here.
7. In the **Input Metadata** section, you can fill in the metadata so that you have all needed information when you are ready to submit genomes to NCBI or GISAID.

8. **Pre-processing** (Data QC) is turned **ON** by default and uses ***FaQCs***. This includes trimming low quality regions of reads and filtering reads that either fail a quality threshold or minimum length. If you wish to change parameters, you can expand the module by clicking on it and modify as desired. The default parameters are as follows:
    - Trim Quality Level: 20 (Illumina), 7(Nanopore)
    - Minimum Read Length: 50 (Illumina), 350(Nanopore)
    - "N" Base Cutoff: 10
    - Low Complexity Filter: 0.85

9. **Trimming primers from samples sequenced using multiplex amplicon approach.** We provide two options to trim primers if a multiplex amplicon approach such as ARTIC (v1-4) CDC protocols, SWIFT protocols, Freed (Midnight) protocols, HiFiViral(Pacbio) and Varskip were used. Default approach is to use the *align_trim,* that soft clips primer region from the alignment file (BAM) based on the position of primers in the reference genome. Another approach is to use FaQC, which trims the regions from reads that match with primer sequences.



10. For samples with whole genome sequencing (WGS) data that are interested in *de novo* assembly, you can also turn on `**Assembly and Annotation**`. Currently we provide IDBA_UD v1.1.1, SPAdes v3.13.0, MEGAHIT v1.2.9, UniCycler v0.4.8, wtdbg2 v2.5, flye v2.8 and miniasm v0.3 as options for assemblies.

11. **Reference-Based SARS-CoV-2 Genome Analysis** is turned **ON** by default. For Illumina data, ***BWA mem*** is used as the default aligner, which is then automatically followed by generation of a consensus sequence and variant calling. For ONT/PacBio data, ***minimap2*** is the default aligner which is also automatically followed by generation of a consensus

sequence, but not variant calling. We currently turn **OFF** variant calling for ONT data as it takes well over 24 hours on this platform. However, you can change any of these parameters by expanding the module and selecting the desired changes. Additionally, to avoid partial short alignment, **samclip** script with *max clip* length 50 for Illumina and 150 for ONT/PacBio is also applied.

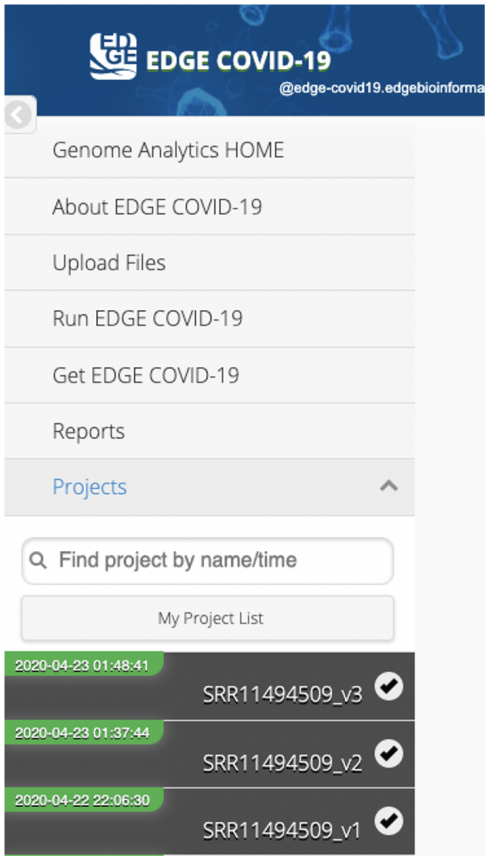
Variant calling: *EDGE COVID-19* uses **bcftools** *mpileup* command to convert the aligned BAM file into genomic positions and call genotypes, reduce the list of sites to those found to be variants by passing this file into **bcftools** *call* command. The variant calls are filtered further by vcfutils.pl of SAMtools with following criteria:

- Minimum Root Mean Square (RMS) mapping quality for SNPs [10];
- minimum read depth [5];
- maximum read depth [1000];
- minimum number of alternate bases [3];
- minimum ratio of alternate bases [0.3];
- SNP within INT bp around a gap to be filtered [3];
- 'window size for filtering adjacent gaps [10];
- min P-value for end distance bias [1e-15];
- maximum fraction of reads supporting an indel [0.5];

The consensus workflow: For samples sequenced using non-amplicon methods, PCR deduplication is also performed. Various parameters are defaulted including a minimum of 5x depth coverage of support or variant siter coverage per base (otherwise the consensus will be "N"), base quality (<20 for Illumina and <5 for ONT), alternate base Threshold (0.5 to support an alternative for the consensus to be changed), indels Threshold (to support an INDEL for the consensus to be changed, 0.5 for illumina and 0.6 for amplicon-based ONT), and minimum mapping quality of 60. The strand bias and homopolymer were checked and filtered for samples from ONT..

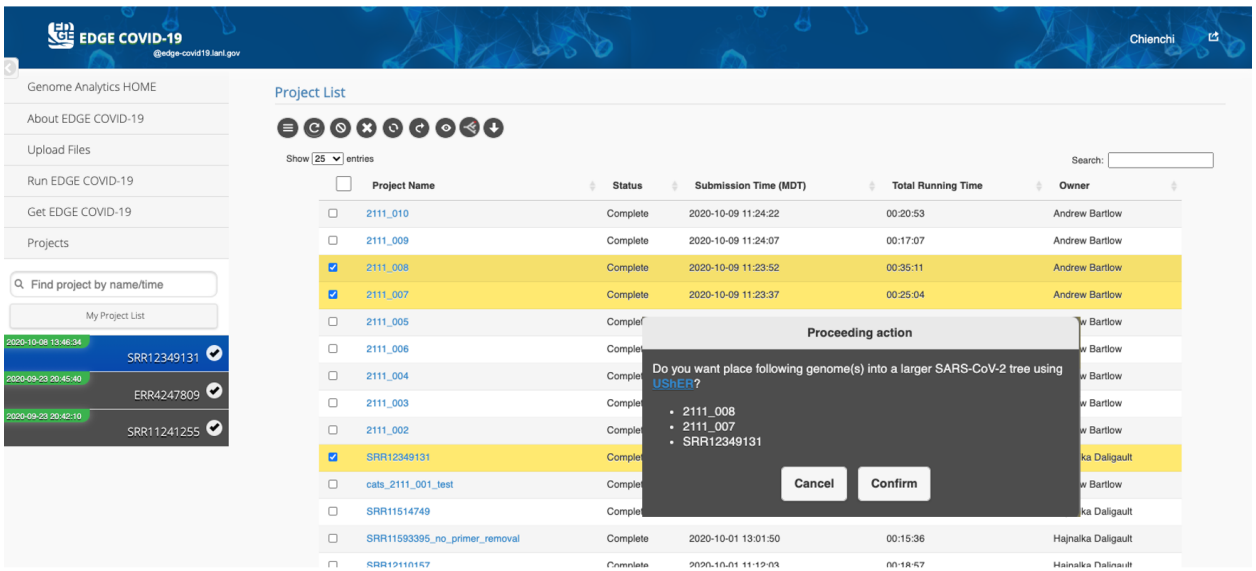
12. Click the **submit** button at the bottom of the page to start your job

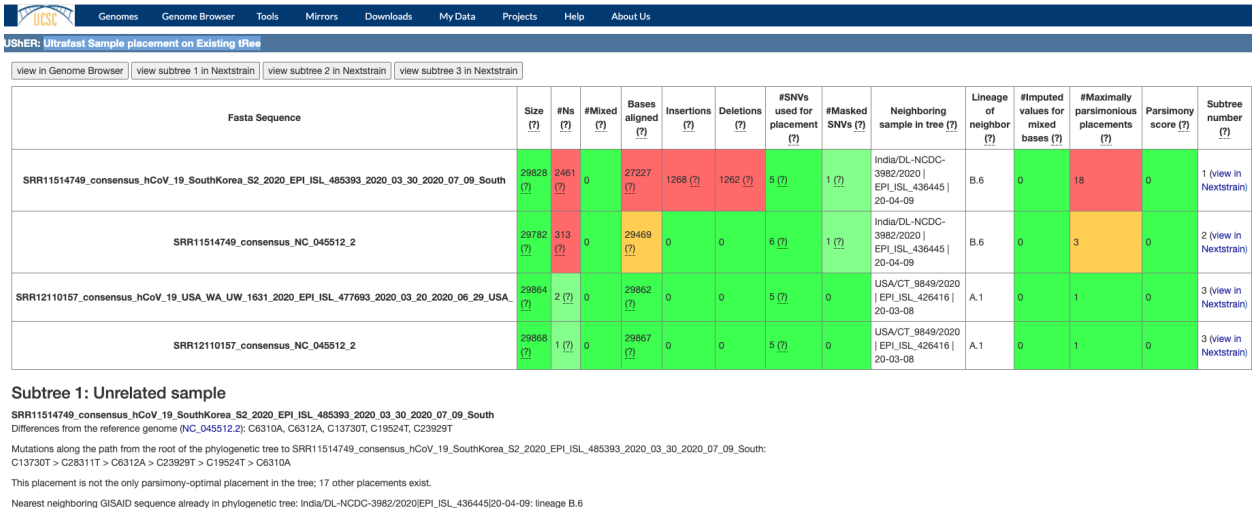
13. The status of all of your projects can be viewed by clicking the **Projects** tab on the left menu and then clicking on **My Project List**. A detailed description can also be found here.



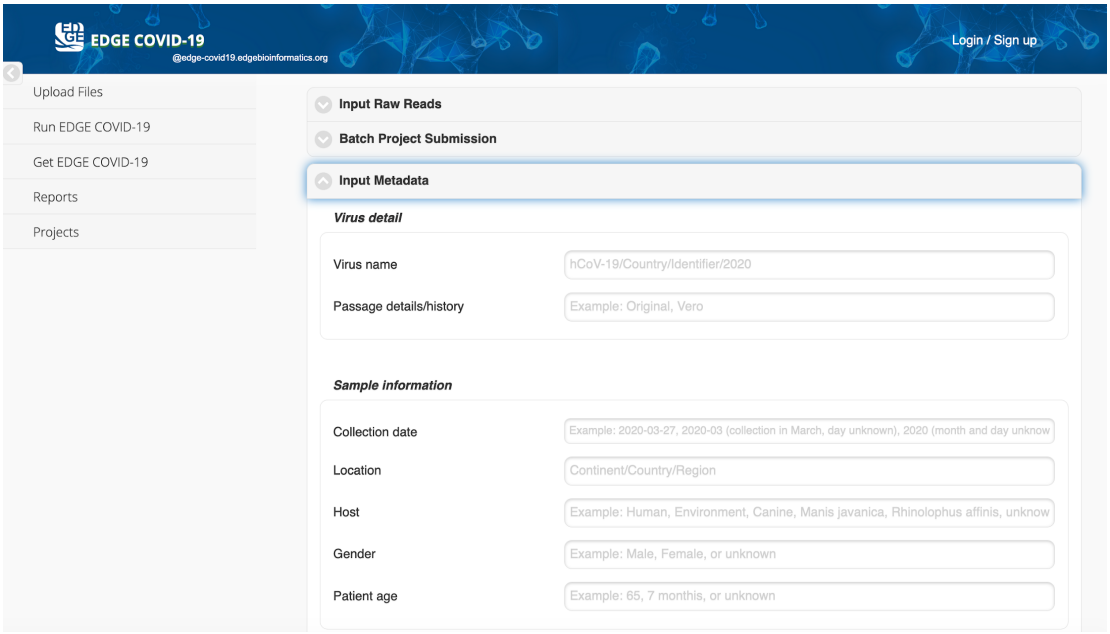
If a project is highlighted in **green** it means the project has finished; if **orange**, **red**, or **grey** (not shown) it means the project is running, cancelled/failed, or not yet started, respectively. You can access the details and outputs of each project by clicking on the project in the list. Once selected, the project results page displays a summary of the run and general statistics of what tools and modules were activated and their runtime and status, as well as links to log files that provide detailed information on the command lines and parameters used for each tool executed. The rest of the page is divided by the modules that were originally selected for analysis. When selecting a project which has not finished running, some of the completed results may still be viewed on the page, however graphics and links to interactive features will not be present, as the rendering of figures is performed only in the last step.

14. **Tree Placement of consensus genome(s) by UShER (Ultrafast Sample placement on Existing Tree)**: you can select the consensus genomes from the project list to do tree placement and output to a new tab with UShER result (link to UCSC).





15. Before submitting the pipeline run, you can prepare your genome for submission to GISAID and NCBI by inputting the metadata. You can do this after the pipeline is run as well.



After entering the metadata, the genome can be submitted to GISAID and NCBI directly through the *EDGE COVID-19* platform. You can access this functionality by clicking on the green check mark in the Reference-based results just below “Ready to Submit”.

Reference-Based SARS-CoV-2 Genome Analysis

a. Reads Mapped to Reference(s)

i. Mapped Reads By bwa

Columns...

SARS-CoV2 Reference	Ref Length	Ref GC%	Mapped Reads	Mapped Reads%	Base Coverage	Avg Fold	Bam File
NC_045512.2	29,870	38.01%	119,708	99.04%	99.97%	725.92X	

Link to I Directory

1 out of 1 reference(s) is(are) covered by input reads.

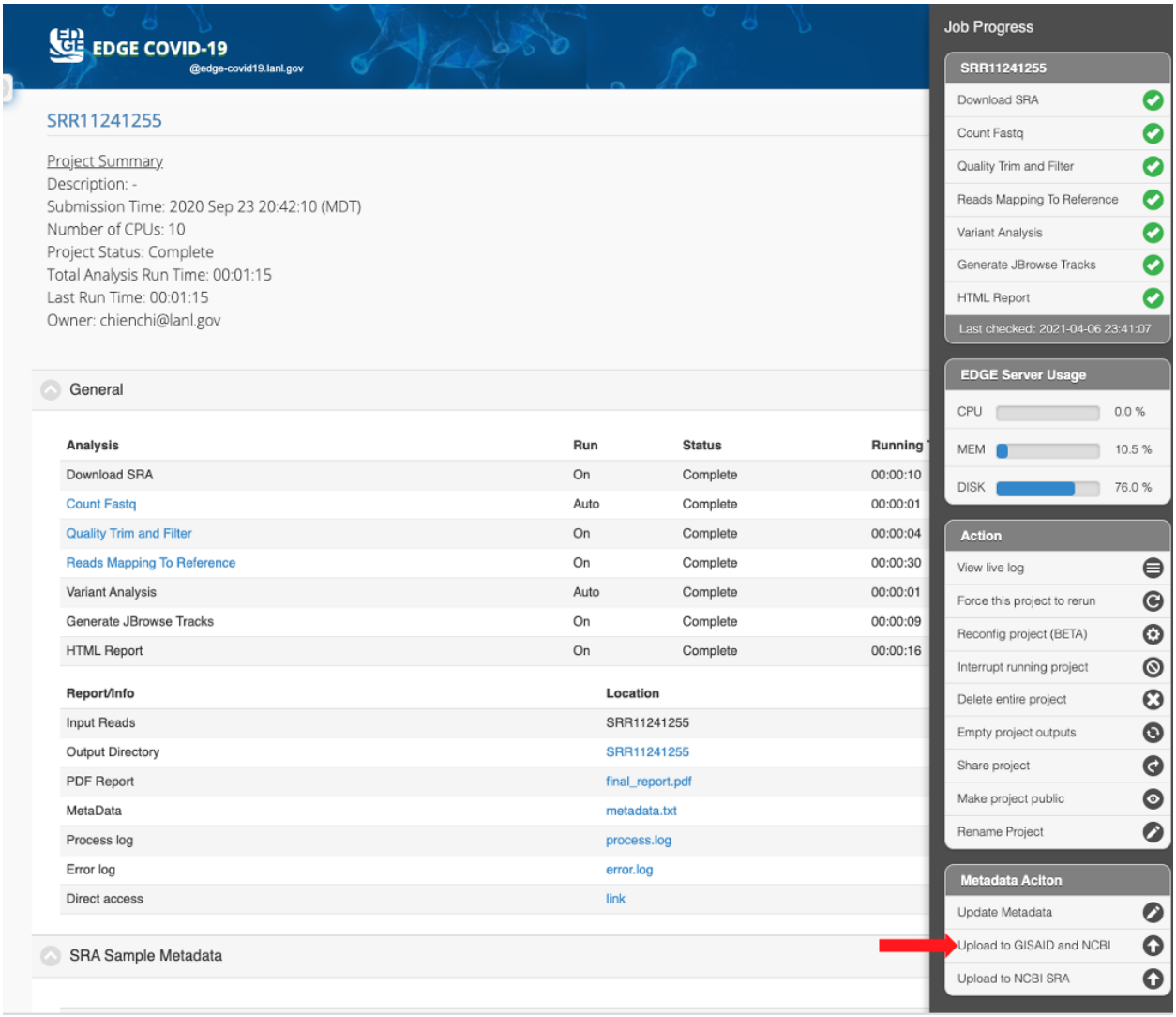
ii. Consensus Genome Statistics

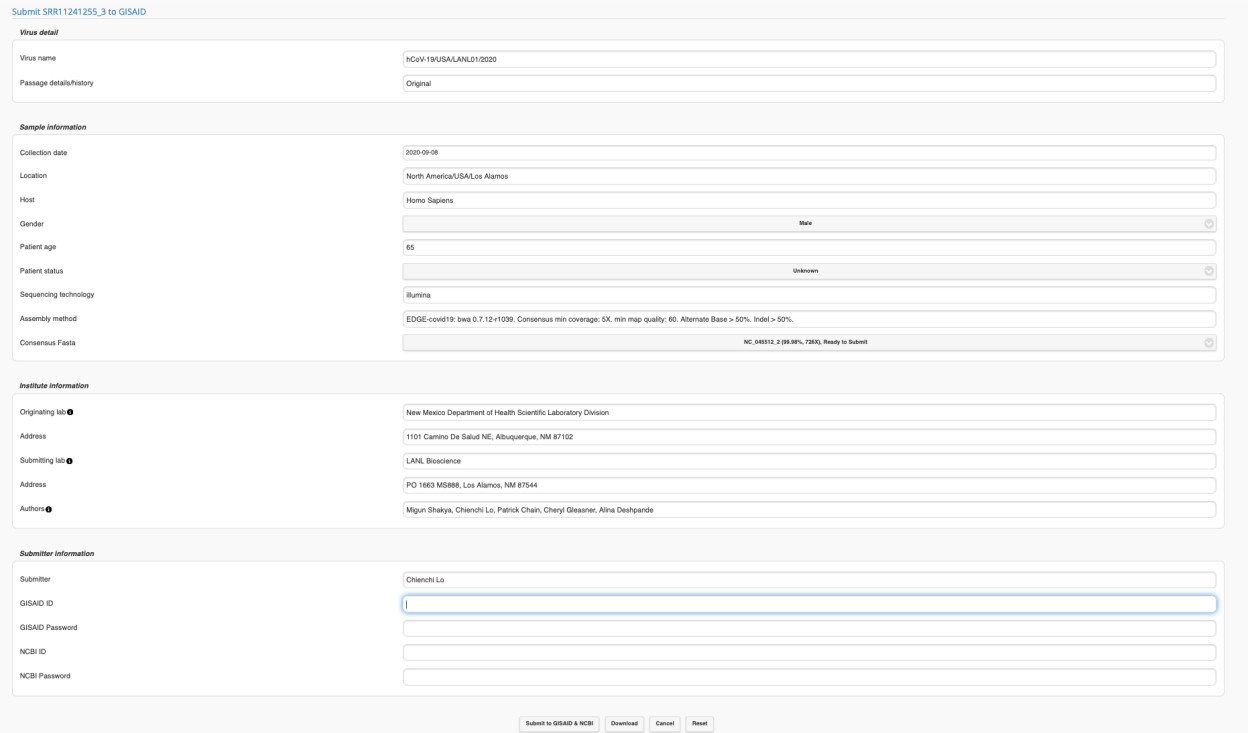
Columns...

SARS-CoV2 Reference	Consensus Length	Gaps	Ns/ns	5' Ns/ns	3' Ns/ns	SNVs	INDELs	Lineage	Consensus Genome	Ready to Submit
NC_045512.2	29,870	1	88	82	6	5	0	A.1		

Link to I Directory

A menu will appear on the right side of the screen. Click the Metadata Action -> Upload to GISAID and NCBI option at the bottom of the menu to submit consensus genomes.





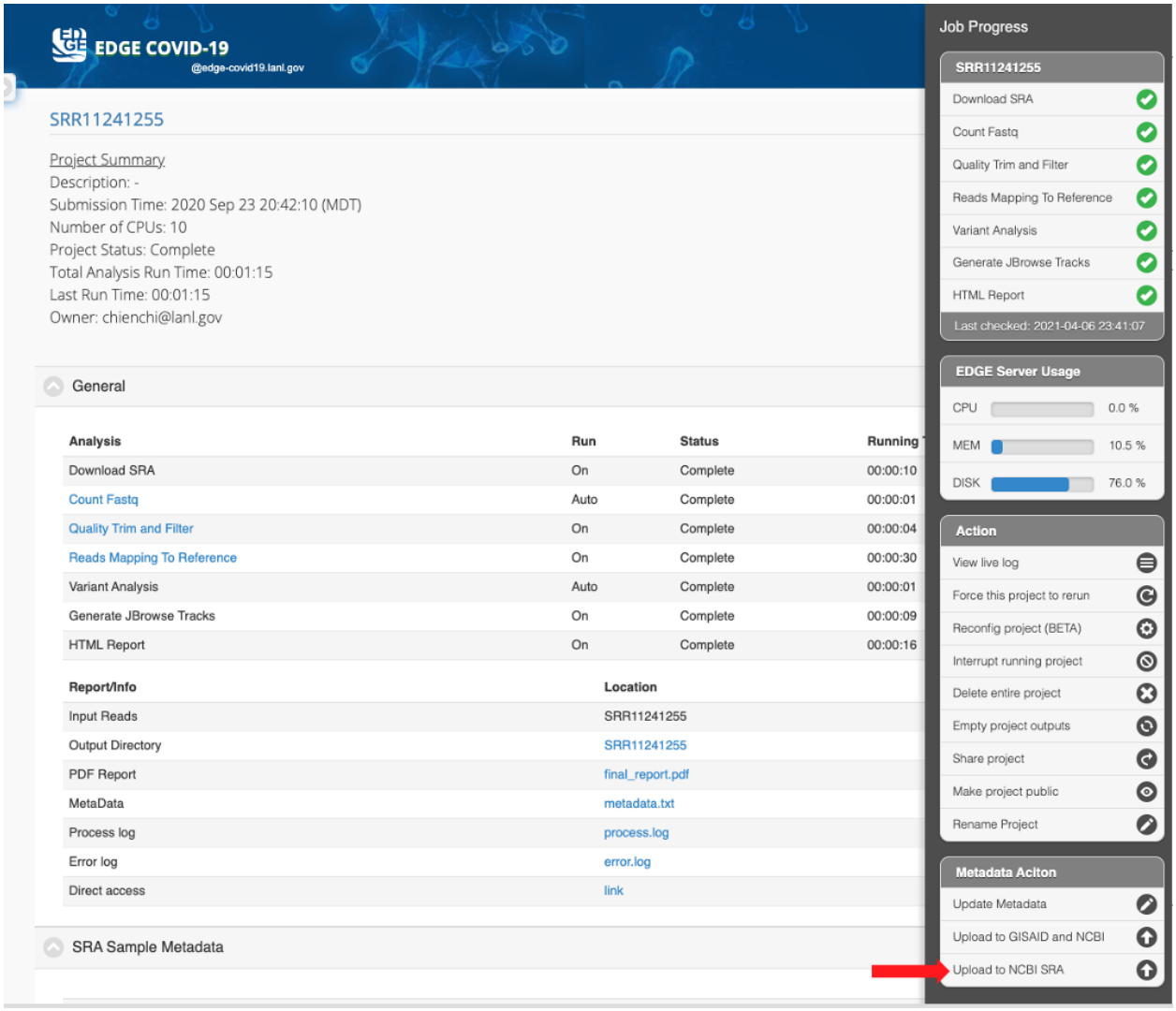
Required metadata fields must be properly filled for submission to proceed. You will need to have a registered GISAID account and a NCBI account.

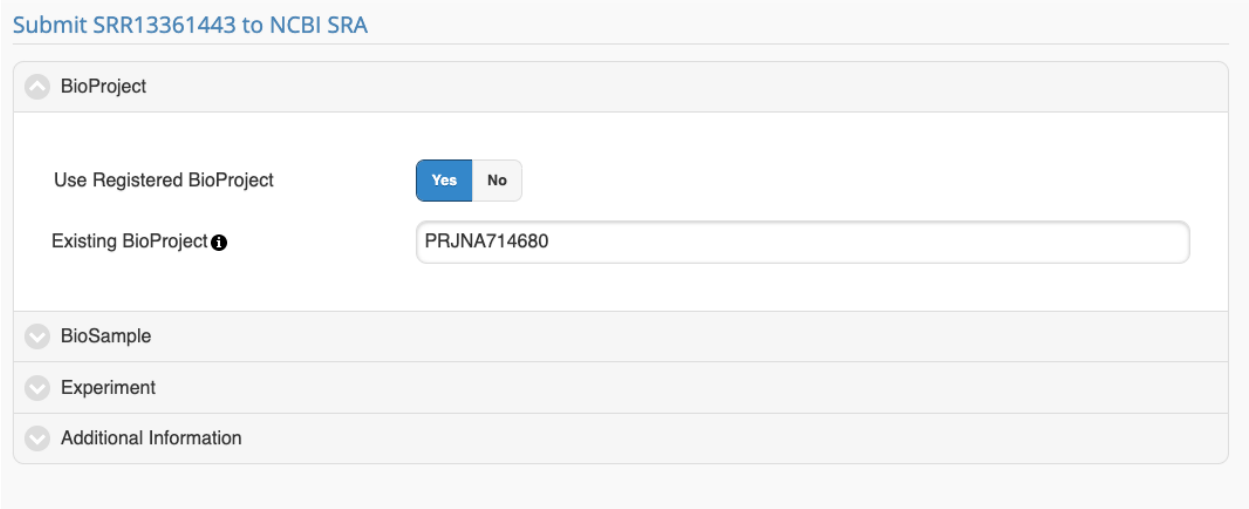
By clicking the "Confirm" button, you hereby authorize EDGE-COVID19 to submit the consensus genomes and metadata to the GISAID and NCBI Genbank, and agree to remit the samples and related metadata to the public domain.

If your submission is successful, you should receive an email from GISAID and NCBI for assigned accession numbers or further instructions.

Note: this feature is in beta format, and GISAID and NCBI can change the submission process at any time; if you run into any trouble, you can contact us at edge-covid19@lanl.gov.

16. Raw Reads submit to NCBI SRA:
In the same menu on the right side of the screen, users can submit the raw reads fastq to NCBI SRA. Click the Metadata Action -> Upload to NCBI SRA option at the bottom of the menu to submit.





Required metadata fields must be properly filled for submission to proceed.

By clicking the "Confirm" button, you hereby authorize EDGE-COVID19 to submit the samples and metadata to the NCBI SRA, and agree to remit the samples and related metadata to the public domain.

If your SRA submission is successful, you should receive an email with the subject *Submission ownership transfer*. After the ownership transfer, you can view the submission process at the Submission Portal. You may need to log in with the NCBI credentials for the account you used in the submission metadata.
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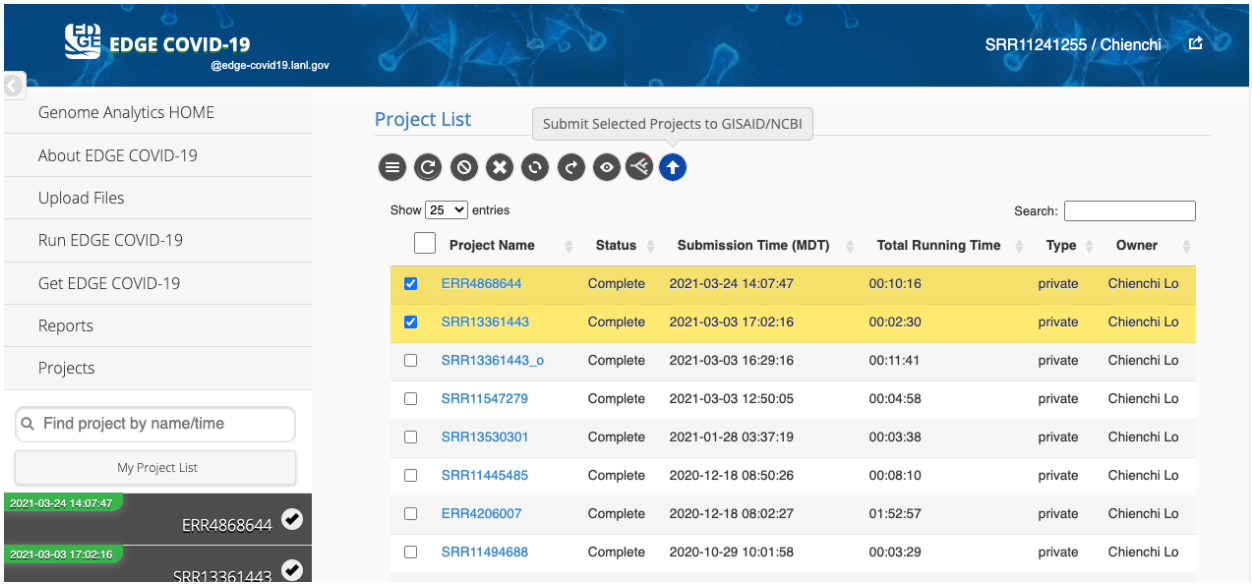
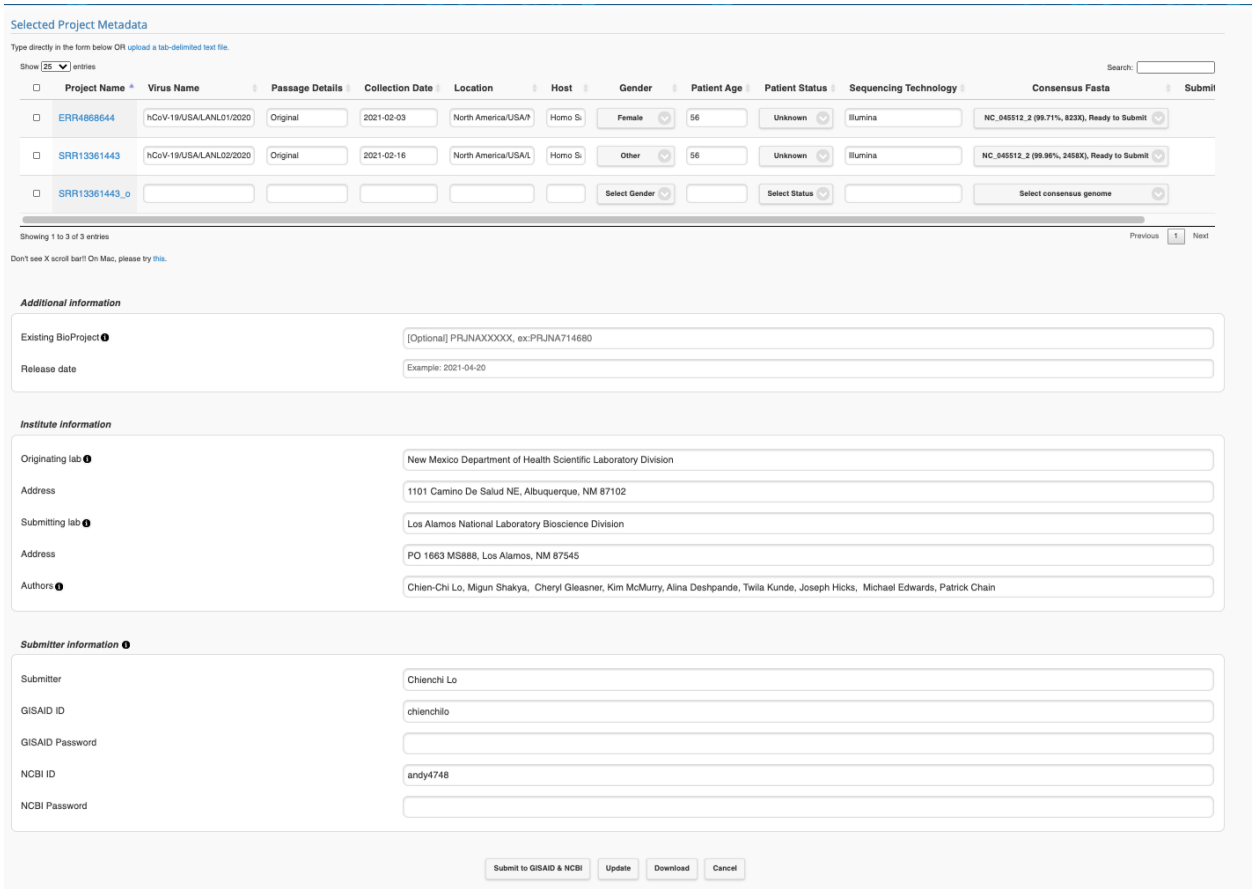
Note: this feature is in beta format, if you run into any trouble, you can contact us at edge-covid19@lanl.gov.

17. Batch submit (consensus genomes):
You can access this functionality by clicking on **My Project List**. Select on projects you would like to do the batch submission and then click on the upper-arrow action button at top of the table.



The action button will bring up the selected projects metadata table for users to fill in. (can scroll to the right to see other metadata.)



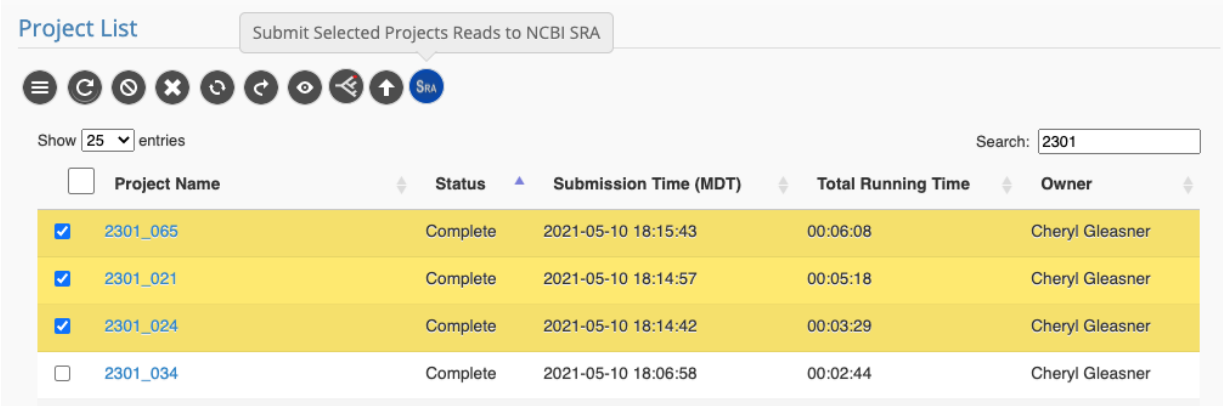
Required metadata fields must be properly filled for submission to proceed. You will need to have a registered GISAID account and a NCBI account.

By clicking the "Confirm" button, you hereby authorize EDGE-COVID19 to submit the consensus genomes and metadata to the GISAID and NCBI Genbank, and agree to remit the samples and related metadata to the public domain.

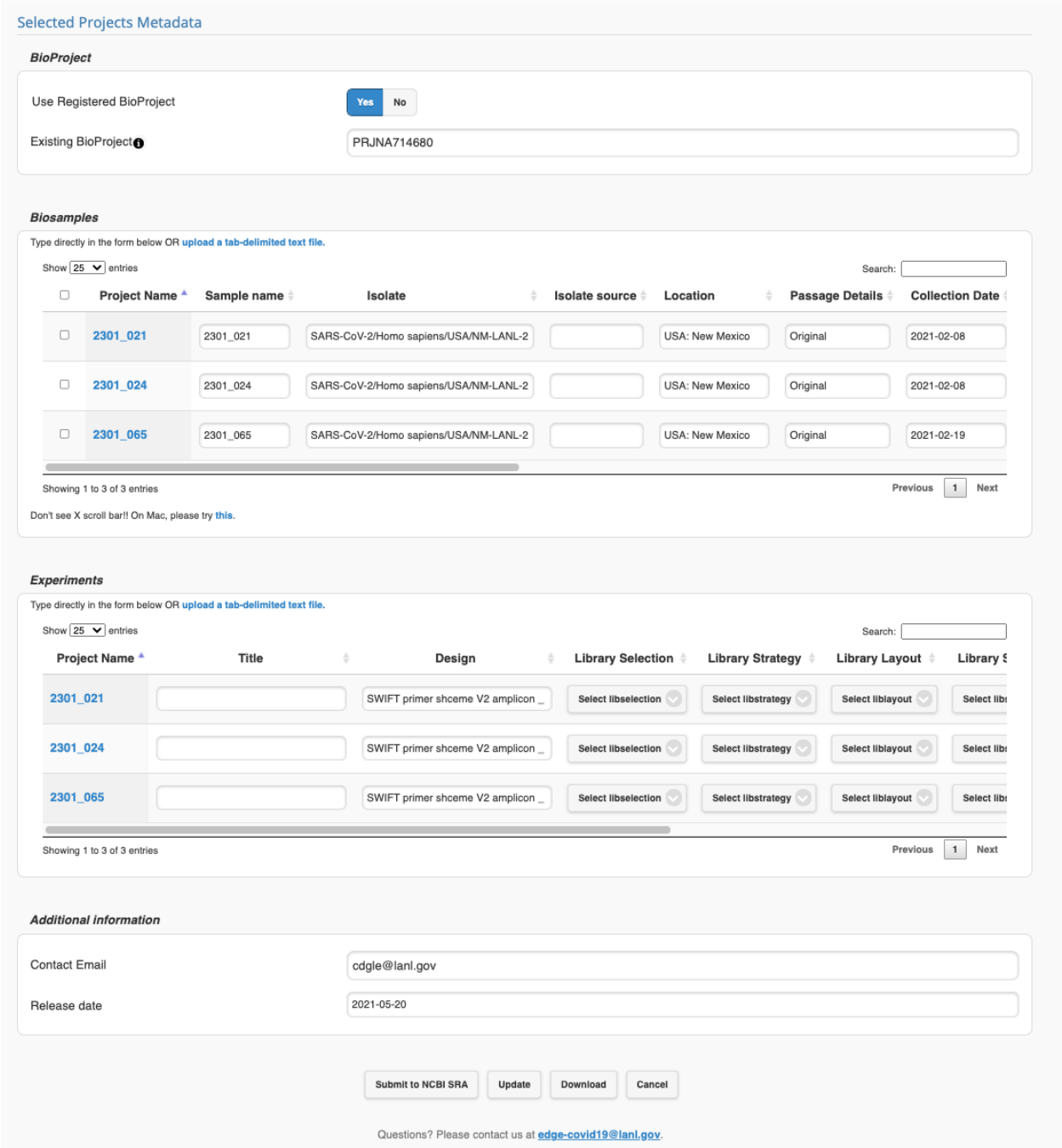
If your submission is successful, you should receive an email from GISAID and NCBI for assigned accession numbers or further instructions.

Note: this feature is in beta format, and GISAID/NCBI can change the submission process at any time; if you run into any trouble, you can contact us at edge-covid19@lanl.gov.

18. Batch submit (NCBI SRA):
You can access this functionality by clicking on **My Project List**. Select on projects you would like to do the batch submission and then click on the right-most (SRA) action button at top of the table.



The action button will bring up the selected projects metadata tables for users to fill in. (can scroll to the right to see other metadata.)



Required metadata fields must be properly filled for submission to proceed.

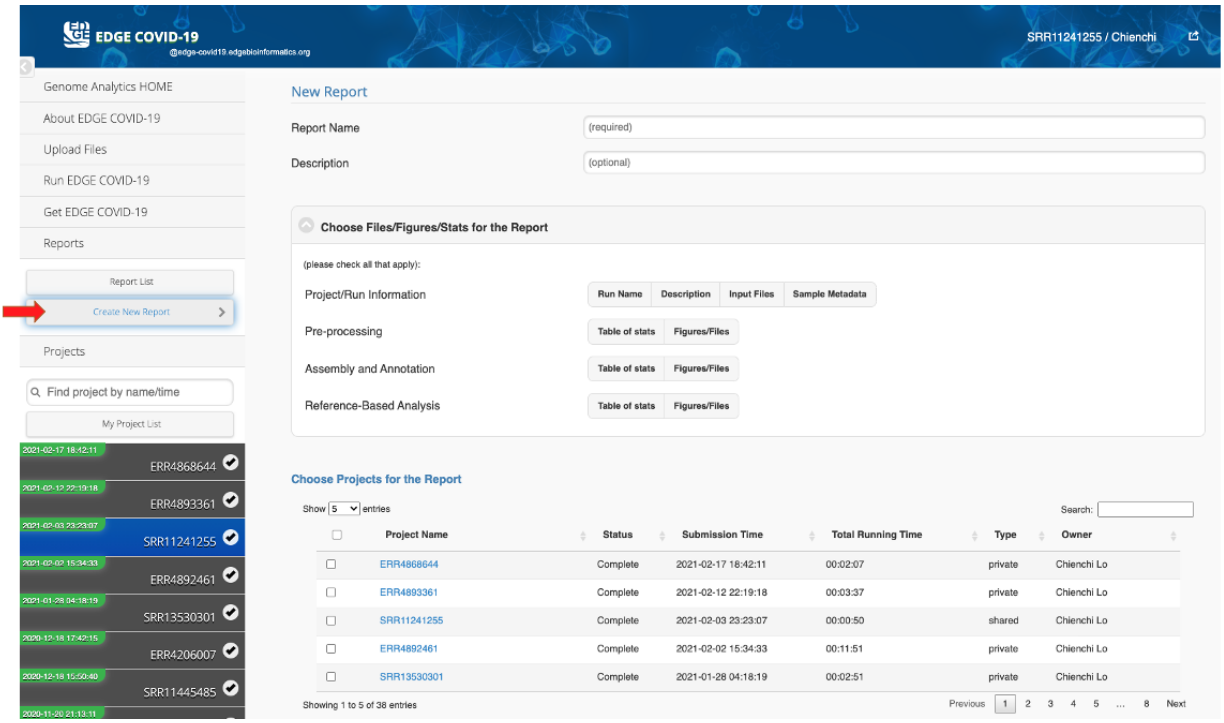
By clicking the "Confirm" button, you hereby authorize EDGE-COVID19 to submit the samples and metadata to the NCBI SRA, and agree to remit the samples and related metadata to the public domain.

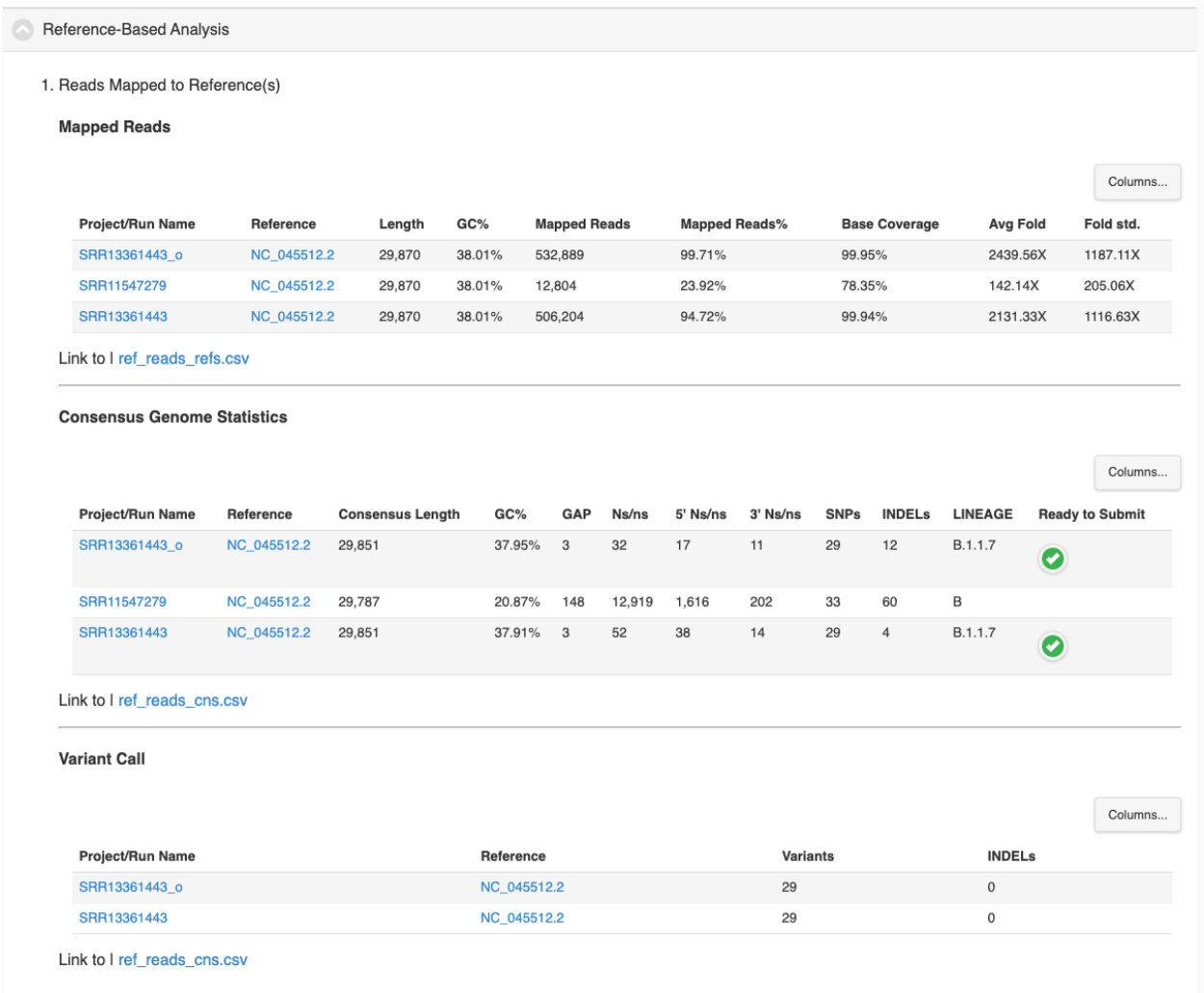
If your SRA submission is successful, you should receive an email with the subject *Submission ownership transfer*. After the ownership transfer, you can view the submission

process at the Submission Portal. You may need to log in with the NCBI credentials for the account you used in the submission metadata.

Note: this feature is in beta format, and NCBI can change the submission process at any time; if you run into any trouble, you can contact us at edge-covid19@lanl.gov.

19. Generate a report that contains comparison among multiple projects.
This feature can be accessed by clicking the “Reports” button on the left side of EC-19 page.





Reference-Based Analysis

1. Reads Mapped to Reference(s)

Mapped Reads

Columns...

Project/Run Name	Reference	Length	GC%	Mapped Reads	Mapped Reads%	Base Coverage	Avg Fold	Fold std.
SRR13361443_o	NC_045512.2	29,870	38.01%	532,889	99.71%	99.95%	2439.56X	1187.11X
SRR11547279	NC_045512.2	29,870	38.01%	12,804	23.92%	78.35%	142.14X	205.06X
SRR13361443	NC_045512.2	29,870	38.01%	506,204	94.72%	99.94%	2131.33X	1116.63X

Link to I ref_reads_refs.csv

Consensus Genome Statistics

Columns...

Project/Run Name	Reference	Consensus Length	GC%	GAP	Ns/ns	5' Ns/ns	3' Ns/ns	SNPs	INDELs	LINEAGE	Ready to Submit
SRR13361443_o	NC_045512.2	29,851	37.95%	3	32	17	11	29	12	B.1.1.7	
SRR11547279	NC_045512.2	29,787	20.87%	148	12,919	1,616	202	33	60	B	
SRR13361443	NC_045512.2	29,851	37.91%	3	52	38	14	29	4	B.1.1.7	

Link to I ref_reads_cns.csv

Variant Call

Columns...

Project/Run Name	Reference	Variants	INDELs
SRR13361443_o	NC_045512.2	29	0
SRR13361443	NC_045512.2	29	0

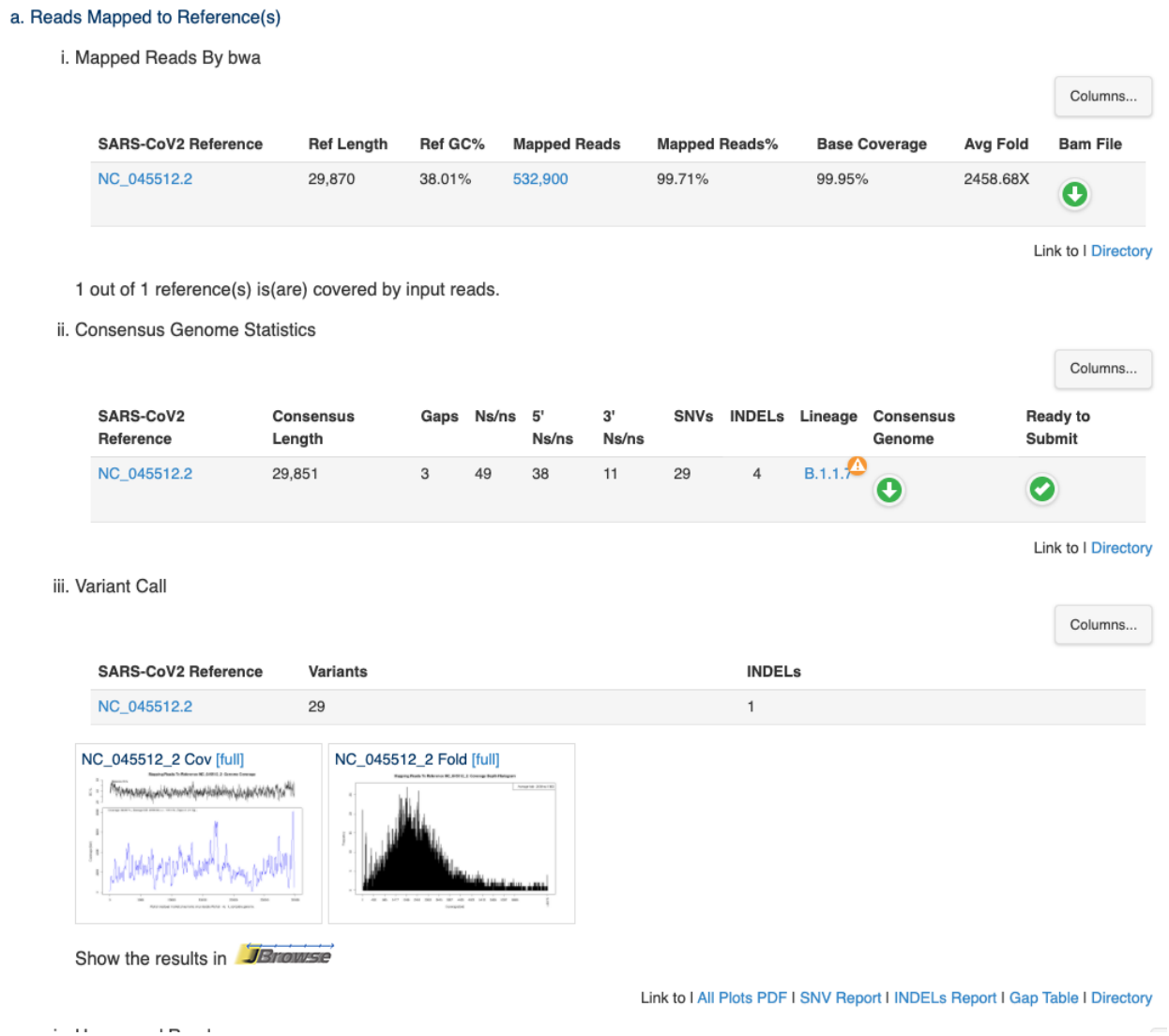
Link to I ref_reads_cns.csv

EDGE COVID-19 output page

For a more detailed description of the EDGE bioinformatics output page, please refer to our full files list in the next section. Each selected module will be displayed as a subsection, and detailed results may be found in each section. The Pre-processing section, for example, will have details on various statistics from all reads both before and after quality trimming and filtering. If assembly/annotation is selected, this module's output will include the assembled contigs as a Fasta file in addition to assembly metrics and annotation files.

In the *EDGE COVID-19* version of **Reference-based SARS-CoV-2 genome analysis**, an overview of the statistics and reference genome coverage is presented, including fold coverage (in graphical form along the length of the reference genome), as well as number of SNPs and gaps discovered (including those at the 5' and 3' ends of the reference genome). The Pangolin (v3) Lineage assignment also reported with hyperlink to outbreak.info for detailed information. A warning icon will be shown if it is a Variant of Concern (VOC) or Variant of Interest (VOI). If any

INDELs cause the frameshift or SNP changes result in early stop codon in the CDS region, a warning icon will be shown too. You can directly download the consensus genome by clicking on the download icon. In our report, we also provide a quality check of the consensus genome by providing a green check mark if the resulting consensus genome is longer than 25kb, has coverage depth greater than 10X, and less than 5% of the genome is Ns. More data such as reference genome, BAM file, etc. can be accessed via the **Directory** link which allows access to all output files (e.g., there is an output file detailing the genomic location of SNPs or variant nucleotides, their prevalence within reads covering that position, any changes in translated amino acid composition, etc.). (See below)



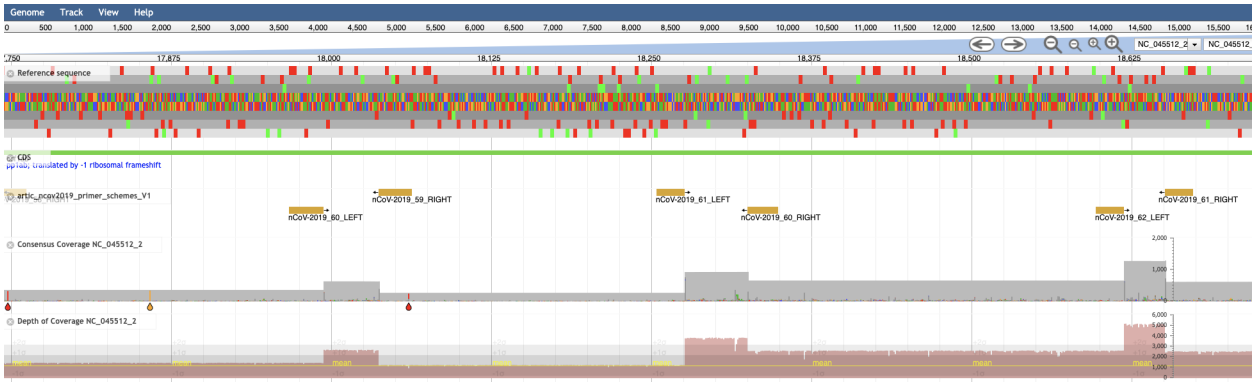
Table

Show 10 entries Search:

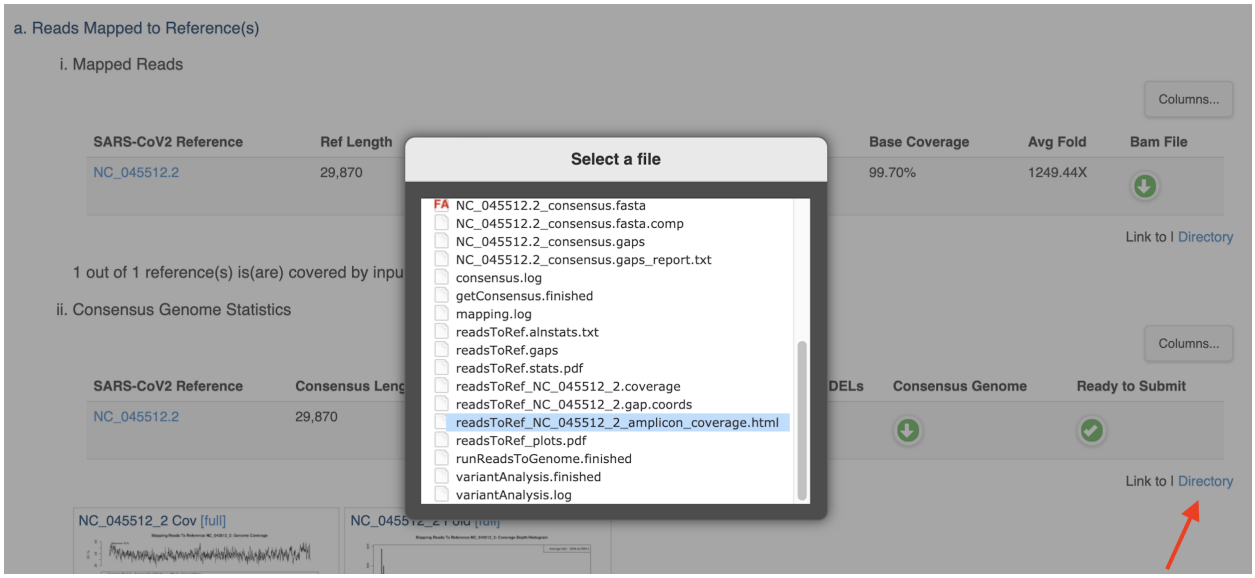
Chromosome	SNP_position	Ref_codon	Sub_codon	aa_Ref	aa_Sub	Synonymous	Product	CDS_start	CDS_end	CDS_stran
NC_045512_2	241	C	T				Intergenic region			
NC_045512_2	913	TCC	TCT	S	S	Yes	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	2110	AAC	AAT	N	N	Yes	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	2836	TGC	TGT	C	C	Yes	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	3037	TTC	TTT	F	F	Yes	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	3267	ACT	ATT	T	I	T1001I	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	6954	ATA	ACA	I	T	I2230T	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	7984	GAT	GAC	D	D	Yes	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	10319	CTT	TTT	L	F	L3352F	GU280_gp01:orf1a polyprotein	266	13483	+
NC_045512_2	14120	CCA	CTA	P	L	P218L	GU280_gp01:orf1ab polyprotein	13468	21555	+

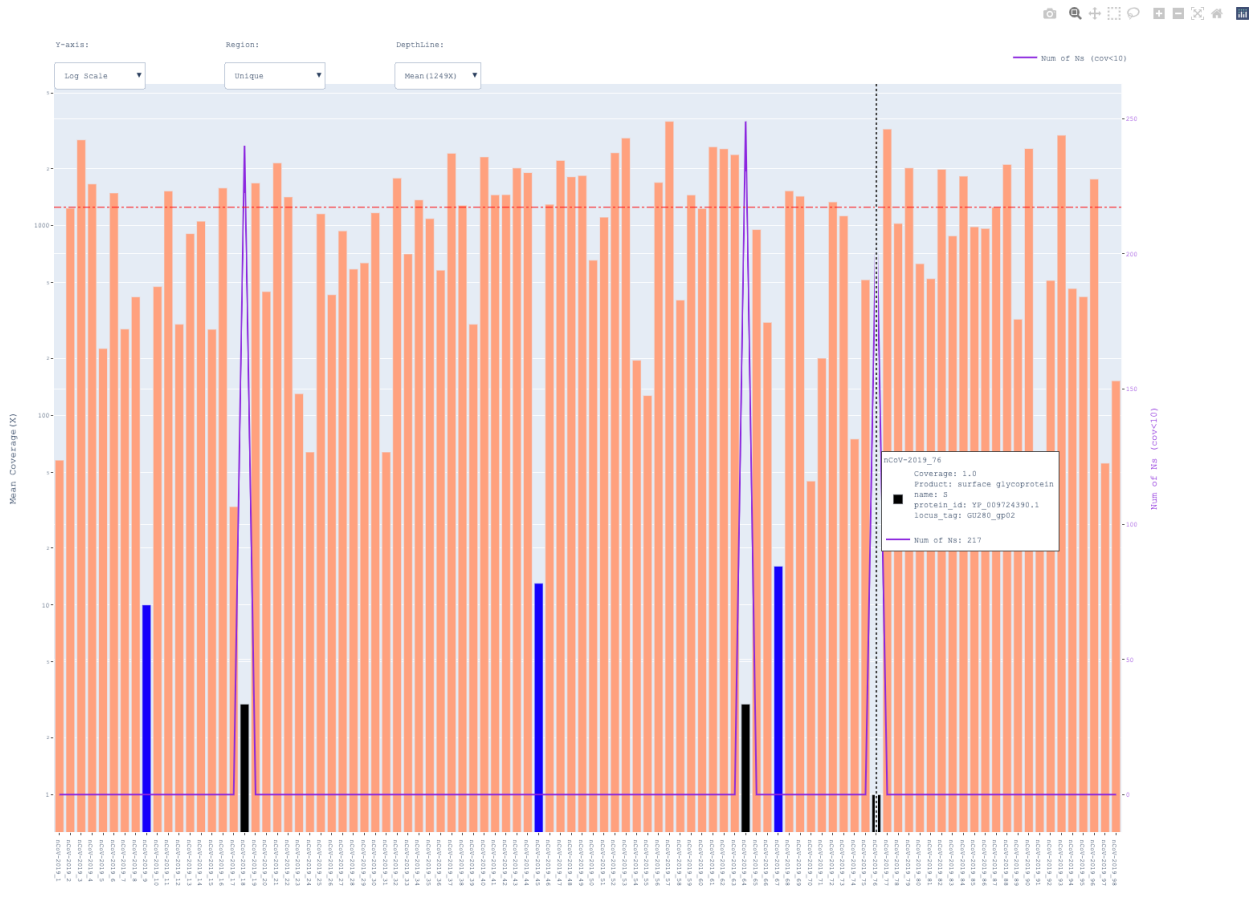
Showing 1 to 10 of 31 entries Previous 1 2 3 4 Next

For scientists wishing to examine the details underlying the statistics, a JBrowse link is also provided (right below the graphics), which will open another browser window and allows interactive examination of the reference genome alignment results including annotations, locations of SNPs or variants, and read alignments (See below).



If the primer ***align_trim*** option has been used, users can also access the amplicon coverage plot in the output **Directory** and clicking the *readsToRef_NC_045512_2_amplicon_coverage.html* will open the graphics in a new browser window (See below).


a. Reads Mapped to Reference(s)
i. Mapped Reads
Columns...
SARS-CoV2 Reference
Ref Length
Base Coverage
Avg Fold
Bam File
NC_045512.2
29,870
99.70%
1249.44X
Link to l Directory
1 out of 1 reference(s) is(are) covered by inpu
ii. Consensus Genome Statistics
Columns...
SARS-CoV2 Reference
Consensus Leng
DELs
Consensus Genome
Ready to Submit
NC_045512.2
29,870
Link to l Directory
Select a file
NC_045512.2_consensus.fasta
NC_045512.2_consensus.fasta.comp
NC_045512.2_consensus.gaps
NC_045512.2_consensus.gaps_report.txt
consensus.log
getConsensus.finished
mapping.log
readsToRef.alnstats.txt
readsToRef.gaps
readsToRef.stats.pdf
readsToRef_NC_045512_2.coverage
readsToRef_NC_045512_2.gap.coords
readsToRef_NC_045512_2_amplicon_coverage.html
readsToRef_plots.pdf
runReadsToGenome.finished
variantAnalysis.finished
variantAnalysis.log
NC_045512_2 Cov [full]


Y-axis:
Region:
DepthLine:
Log Scale
Unique
Mean(1249X)
Num of Ns (cov<10)
Mean Coverage(X)
Num of Ns (cov<10)
nCoV-2019_76
Coverage: 1.0
Product: surface glycoprotein
name: S
protein_id: YP_009724390.1
locus_tag: GU280_gp02
Num of Ns: 217

EDGE COVID-19 output files

* **Bold Files** are files with easy to download buttons in the project result page of EDGE GUI. For advanced users, other files are accessible using the GUI project file browser (**Directory**) in the result page. The directory is in a grey background.

Project	File Descriptions
├ batch_input.json	batch input parameters
├ clusterJob.log	cluster submit ID and log
├ clusterSubmit.sh	cluster submit shell script
├ config.json	EDGE configuration in JSON
├ config.txt	EDGE configuration in text
├ error.log	project error log
├ final_report.pdf	result pdf
├ HTML_Report	result HTML directory
├ JBrowse	JBrowse tracks directory
├ metadata_gisaid_ncbi.txt	project sample metadata for gisaid and ncbi
├ metadata_run.txt	project run ID
├ process_current.log	project last run log
├ process.log	project overall process log
├ QcReads	QC processed directory
│ ├ all.1.fastq	input forward reads
│ ├ all.2.fastq	input reverse reads
│ ├ fastqCount.txt	stats for fastq count
│ ├ QC.1.trimmed.fastq	trimmed forward reads
│ ├ QC.2.trimmed.fastq	trimmed reverse reads

│ ├ QC.log	QC log
│ ├ QC_qc_report.pdf	QC stats report pdf
│ ├ QC.stats.txt	QC stats text
│ ├ QC.unpaired.trimmed.fastq	trimmed single end/orphan reads
├ ReadsBasedAnalysis	
│ └── readsMappingToRef	
│ ├ AlignTrimMapping/	Align Trim reads fastq directory
│ ├ consensus.log	consensus workflow log
│ ├ Coverage_plots/	reference covreage and histogram png files directory
│ ├ GapVSReference.report.json	Gap analysis result json file
│ ├ GapVSReference.report.txt	report of All reference genes affacted by gap regions
│ ├ mapping.log	reads mapping log
│ ├ NC_045512.2.alnstats.txt	reads mapping to NC_045512 stats
│ ├ NC_045512.2_consensus.changelog	consensus workflow nucleotide changes info
│ ├ **NC_045512.2_consensus.fasta ***	consensus fasta file
│ ├ **NC_045512.2_consensus_w_ambiguous.fasta ***	consensus fasta file with IUPAC code
│ ├ NC_045512.2_consensus.fasta.comp	consensus fasta nucleotide composition
│ ├ NC_045512.2_consensus_w_ambiguous.fasta.comp	consensus fasta with IUPAC code nucleotide composition

│ ├ NC_045512.2_consensus.gaps	no reads mapped to NC_045512 regions
│ ├ NC_045512.2_consensus.gaps_report.txt	report of NC_045512 genes affacted by gap regions
│ ├ NC_045512.2_consensus.Indels_report.txt	report of NC_045512 genes affacted by INDELs
│ ├ NC_045512.2_consensus_lineage.txt	report of Pangolin lineage assignment
│ ├ NC_045512.2_consensus.SNPs_report.txt	report of NC_045512 genes affacted by SNPs
│ ├NC_045512.2_consensus_w_ambiguous.SNPs_report.txt	report of NC_045512 genes affacted by SNPs with IUPAC code
│ ├ **NC_045512.2.sort.bam ***	reads mapping bam file
│ ├ NC_045512.2.sort.bam.bai	reads mapping bam index file
│ ├ NC_045512.2.vcf	variant call of reads mapping to NC_045512 result by bcftools
│ ├ pangolin.log	Pangolin lineage run log
│ ├ readsToRef.alnstats.txt	reads mapping to All reference stats
│ ├ readsToRef.gaps	reads mapping to All reference gaps
│ ├ readsToRef.Indels_report.txt	report of All reference genes affacted by INDELs
│ ├ readsToRef_NC_045512_2_amplicon_coverage.html	amplicon coverage interactive plot
│ ├ readsToRef_NC_045512_2_amplicon_coverage.txt	amplicon coverage based on primer scheme bed file
│ ├ readsToRef_NC_045512_2.coverage	NC_045512 genome coverage per base position

│ ├ readsToRef_plots.pdf	coverage plots of reads mapping to all reference
│ ├ readsToRef.SNPs_report.txt	report of All reference genes affacted by SNPs
│ ├ readsToRef.vcf	variant call of reads mapping to all reference result by bcftools
│ ├ swift_primer_schemes_v2.bed	bed file for primer trimming and amplicon coverage plot
│ └── variantAnalysis.log	variants gene analysis
├ Reference	
│ ├ NC_045512.2.fasta	input reference fasta
│ ├ reference.fasta	all input reference fasta
│ ├ reference.gbk	all input reference genbank
│ ├ reference.gff	all input reference gff (convert from gbk)
│ └── ref_list.txt	the accesion list of input reference
├ ReferenceBasedAnalysis	
│ └── readsMappingToRef	symlink direcotry from ReadsBasedAnalysis
└── UPLOAD	
├ sra_experiments.txt	metadata for SRA submission
└── sra_samples.txt	metadata for SRA submission

ThirdParty Tools

- Alignment

- Bowtie 2
  - Citation: Langmead, B. and Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2, Nature methods, 9, 357-359.
  - Site: http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
  - Version: 2.4.1
  - License: GPLv3
- BWA
  - Citation: Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform, Bioinformatics, 25, 1754-1760.
  - Site: http://bio-bwa.sourceforge.net/
  - Version: 0.7.12
  - License: GPLv3
- minimap2
  - Citation: Li, H. (2018) Minimap2: fast pairwise alignment for nucleotide sequences. Bioinformatics, 34:3094-3100.
  - Site: https://github.com/lh3/minimap2
  - Version: 2.17
  - License: MIT
- Kallisto
  - Citation: Nicolas L Bray, et al. (2016) Near-optimal probabilistic RNA-seq quantification, Nature Biotechnology 34, 525–527
  - Site: https://pachterlab.github.io/kallisto/
  - Version: 0.46.0
  - License: BSD 2-Clause

- Annotation
  - RATT
    - Citation: Otto, T.D., et al. (2011) RATT: Rapid Annotation Transfer Tool, Nucleic acids research, 39, e57.
    - Site: http://ratt.sourceforge.net/
    - Version:
    - License: GPLv3

        - Note: **The original RATT program does not deal with reverse complement strain annotations transfer. We edited the source code to fix it.**
- Assembly
    - IDBA-UD
        - Citation: Peng, Y., et al. (2012) IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth, Bioinformatics, 28, 1420-1428.
        - Site: http://i.cs.hku.hk/~alse/hkubrg/projects/idba_ud/
        - Version: 1.1.1
        - License: GPLv2
    - SPAdes
        - Citation: Nurk, Bankevich et al. (2013) Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol. 2013 Oct;20(10):714-37
        - Site: http://bioinf.spbau.ru/spades
        - Version: 3.13.0
        - License: GPLv2
    - MEGAHIT
        - Citation: Li D. et al. (2015) MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015 May 15;31(10):1674-6
        - Site: https://github.com/voutcn/megahit
        - Version: 1.2.9
        - License: GPLv3
    - LRASM: Long Read Assembler
        - Citation:
        - Site: https://gitlab.com/chienchi/long_read_assembly
        - Version: 0.1.0
        - License: GPLv3
    - RACON

        - Citation: Vaser R et al.(2017) Fast and accurate de novo genome assembly from long uncorrected reads. Genome Res. 2017 May;27(5):737-746.
        - Site: https://github.com/isovic/racon
        - Version: 1.4.13
        - License: MIT
    - Unicycler
        - Citation: Wick RR et al.(2017) Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017 Jun 8;13(6):e1005595.
        - Site: https://github.com/rrwick/Unicycler
        - Version: 0.4.8
        - License: GPLv3
- Lineage Assignment
    - Pangolin
        - Citation:Andrew Rambaut et al. (2020) A dynamic nomenclature proposal for SARS-CoV-2 to assist genomic epidemiology. Nat Microbiol. 2020 Nov;5(11):1403-1407.
        - Site: https://pangolin.cog-uk.io/
        - Version: 4.0.1
        - License: GPLv3
- Reads Quality Control
    - FaQCs
        - Citation: Chienchi Lo, PatrickS.G. Chain (2014) Rapid evaluation and Quality Control of Next Generation Sequencing Data with FaQCs. BMC Bioinformatics. 2014 Nov 19;15
        - Site: https://github.com/LANL-Bioinformatics/FaQCs
        - Version: 2.09
        - License: GPLv3
    - NanoPlot
        - Citation: De Coster W, et al.(2018) NanoPack: visualizing and processing long read sequencing data, Bioinformatics. 2018 Mar 14.
        - Site: https://github.com/wdecoster/NanoPlot

        - Version: 1.13.0
        - License: GPLv3
    - Porechop
        - Citation: Wick RR, Judd LM, et al (2017). Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genom. 2017;3(10):e000132. Published 2017 Sep 14. doi:10.1099/mgen.0.000132
        - Site: https://github.com/rrwick/Porechop
        - Version: 0.2.3
        - License: GPLv3
    - Align Trim
        - Site: https://github.com/artic-network/fieldbioinformatics/blob/master/artic/align_trim.py
        - Version:
        - License: MIT
        - Note: **The original Align Trim script does not deal with illumina reads and strandness. We edited the source code to work on it.**
- Utility
    - R
        - Citation: R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
        - Site: http://www.r-project.org/
        - Version: 3.6.3
        - License: GPLv2
    - GNU_parallel
        - Citation: O. Tange (2011): GNU Parallel - The Command-Line Power Tool, ;login: The USENIX Magazine, February 2011:42-47
        - Site: http://www.gnu.org/software/parallel/
        - Version: 20190422
        - License: GPLv3
    - Seqtk

        - Citation: Heng Li https://github.com/lh3/seqtk
        - Site: https://github.com/lh3/seqtk
        - Version: 1.3
        - License: MIT
    - sratoolkit
        - Citation:
        - Site: https://github.com/ncbi/sra-tools
        - Version: 2.9.6
        - License: Public Domain
    - ea-utils
        - Citation: Erik Aronesty (2011) ea-utils : “Command-line tools for processing biological sequencing data”
        - Site: https://code.google.com/archive/p/ea-utils/
        - Version: 1.1.2-537
        - License: MIT License
    - Anaconda3 (Python 3)
        - Citation:
        - Site: https://anaconda.org
        - Version: 2020.02
        - License: 3-clause BSD
- Variants Calling
    - SAMtools
        - Citation: Li, H., et al. (2009) The Sequence Alignment/Map format and SAMtools, Bioinformatics, 25, 2078-2079.
        - Site: http://www.htslib.org/
        - Version: 1.10
        - License: MIT
    - BCFtools
        - Citation: Heng Li (2011) A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data, Bioinformatics (2011) 27(21) 2987-93.
        - Site: http://www.htslib.org/
        - Version: 1.10

        - License: MIT
- Visualization
    - JBrowse
        - Citation: Skinner, M.E., et al. (2009) JBrowse: a next-generation genome browser, Genome research, 19, 1630-1638.
        - Site: http://jbrowse.org
        - Version: 1.16.8
        - License: Artistic License 2.0/LGPLv.1
    - IGV.js
        - Citation: James T. Robinson, et al. (2020) igv.js: an embeddable JavaScript implementation of the Integrative Genomics Viewer (IGV). bioRxiv 2020.05.03075499.
        - Site: https://igv.org/
        - Version: 2.10.4
        - License: MIT

FAQs

For web based app:

1. ### How can I view alignments in a local viewer such as IGV?

   You can download the BAM file using the green download button from the *Mapped Reads* section and the index file can be downloaded by clicking the hyperlinked **Directory** and then clicking on the index file (.bai).
2. ### Can edge-covid19 handle PacBio data?

   Our QC tool FaQC cannot process the base quality values reported by PacBio Sequel as it reports all base qualities as PHRED 0. We recommend you run QC separately or turn **OFF** the **Preprocessing** module (FaQCs will throw an error due to Quality issue), select **YES** to **Nanopore Reads** in **Input Raw Reads** module, and add "-x map-pb" in the **Aligner Option** field in the additional options of **Reference-Based analysis module**.

For local docker build:

1. How to start/stop EDGE COVID-19 docker instance?

To start or restart *EDGE COVID-19*, you will need to run following command in your Terminal from the same directory:

```
$ cd EDGE-COVID19
$ docker rm -v edge-covid19
$ docker run -d --volumes-from mysql_data \
    -v $PWD/EDGE_output:/home/edge/EDGE_output \
    -v $PWD/EDGE_input:/home/edge/EDGE_input \
    -v $PWD/EDGE_report:/home/edge/EDGE_report \
    -p 80:80 -p 8080:8080 --name edge-covid19 bioedge/edge-covid19
```

Then wait a few minutes and go to http://localhost in your favorite browser.

To stop the docker run following command in your directory:

```
$ docker stop edge-covid19
```

Note that the docker container will keep running in the background until you restart your computer or specifically stop it using the above command.

2. How to update EDGE COVID-19?

To update the image to the latest version, you can pull the docker again in the original `EDGE-COVID19` folder used in **Step 1**.

```
$ docker pull bioedge/edge-covid19
```

After pulling the latest docker, start the image from terminal:

```
$ cd EDGE-COVID19
$ docker rm -v edge-covid19
$ docker run -d --volumes-from mysql_data \
    -v $PWD/EDGE_output:/home/edge/EDGE_output \
    -v $PWD/EDGE_input:/home/edge/EDGE_input \
    -v $PWD/EDGE_report:/home/edge/EDGE_report \
    -p 80:80 -p 8080:8080 --name edge-covid19 bioedge/edge-covid19
```

3. How long will it take to run my sample?

Using a Macbook Pro with 16GB RAM and 8 processors available:

dataset size (bases)	# Raw reads	Type of data (Nanopore/ Illumina)	Protocol	# of CPUs	Total Wall Clock Time
416,793,360	10,493,168	Illumina	Amplicons	4	2:38:01
594,064,863	1,382,016	Nanopore	ARTIC protocol	4	0:21:07

4. I am getting an error while pulling the image. What can I do?

If you have issues pulling this image, you may increase the basesize when launching docker daemon or use a different Storage Driver. See similar issue here.

5. I am getting an error while trying to login on GUI: "Failed to login in. Please check server log for details"

In some linux environments, users need to set the /path/to/mysql directory into 0777 mode.

Please try opening the directory permissions if you run into trouble.

```
  $ docker pull bioedge/edge_ubuntu_mysql
  $ docker create --name mysql_data --volume /var/lib/mysql
bioedge/edge_ubuntu_mysql
  $docker run -d --volumes-from mysql_data \
     -v $PWD/EDGE_output:/home/edge/EDGE_output \
     -v $PWD/EDGE_input:/home/edge/EDGE_input \
     -v $PWD/EDGE_report:/home/edge/EDGE_report \
     -p 80:80 -p 8080:8080 --name edge-covid19 bioedge/edge-covid19
```

6. IP address conflicts

Docker is hard coded to look for 172.17.0.1. If the IP address conflicts with the subnet of your WiFi, you may need to customize the docker bridge by editing the /etc/docker/daemon.json as described here.

7. What are the commands for checking status and error log?

Check the MySQL status in container:

```
$ docker exec edge-covid19 service mysql status
```

where "edge-covid19" is the container name when using `docker run` with --name flag
Check container status.

```
$ docker ps -a
```

Check user management system service status:

```
$ docker exec edge-covid19 service tomcat7 status
```

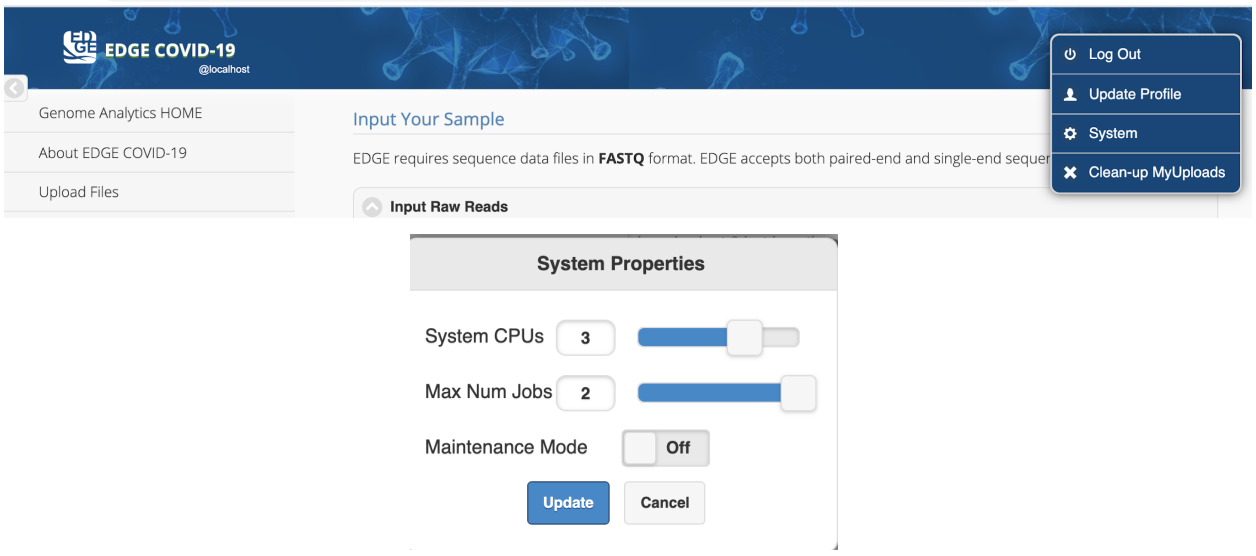
Check the Apache web server status and log:

```
$ docker exec edge-covid19 service apache2 status
$ docker exec edge-covid19 tail /var/log/apache2/error.log
$ docker exec edge-covid19 tail /var/log/apache2/access.log
```

8. How can I update the number of CPUs that EDGE COVID-19 uses?

*

The default number of CPUs available to EDGE inside the container is 4 and the maximum number of jobs can run simultaneously is 2.
Each job will use (*edge_system_cpu-1)/max_num_jobs* in integer CPUs (as you see on the GUI). These numbers can be changed by login using an admin account and click on the user name to pop up the user menu where you can click the **system** button to open the system properties menu.



Contact:

You can view the discussions in the google group below and join the group to post questions and/or comments.

EDGE user's google group at https://groups.google.com/d/forum/edge-users

You can also directly contact us through email at edge-covid19@lanl.gov

Citation:

Chien-Chi Lo, Migun Shakya, Ryan Connor, Karen Davenport, Mark Flynn, Adán Myers y Gutiérrez, Bin Hu, Po-E Li, Elais Player Jackson, Yan Xu, Patrick S G Chain, <b>EDGE COVID-19: A Web Platform to generate submission-ready genomes from SARS-CoV-2 sequencing efforts, Bioinformatics, 2022;, btac176
https://doi.org/10.1093/bioinformatics/btac176