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Abstract

PURPOSE: Step-by-step instructions for submitting pathogen whole genome sequence data to NCBI and to the NCBI Pathogen Detection portal. This protocol covers the steps needed to establish a new NCBI submission environment for your laboratory, including the creation of new BioProject(s) and submission groups. Once these are step up, the protocol then walks through the process for submitting raw reads to SRA and sample metadata to BioSample through the Submission portal.

SCOPE: for use by any laboratory submitting WGS data for species under active surveillance within NCBI's Pathogen Detection. (This includes US laboratories in GenomeTrakr, NARMS, Vet-LIRN, PulseNet, and other non-US networks and submitters).

For new submitters, there's quite a bit of groundwork that needs to be established before a laboratory can start its first data submission. We recommend that one person in the laboratory take a few days to get everything set up in advance of when you expect to do your first data submission.

If you need a pipeline for frequent or large volume submissions, follow Step 1 to get your NCBI submission environment established, then contact **gb-admin@ncbi.nlm.nih.gov** to set up an account for submitting through the API.

This protocol covers submission using NCBI's Submission Portal web-interface.

Version history:

V4: updated screenshots to reflect NCBI submission portal changes. Updated custom BioSample template.

V5: Linking directly to the metadata template guidance instead of including duplicate copies of the files in this protocol. Updated screenshot for choosing the pathogen template to reflect changes at NCBI.

V6: minor edits including updating links out to other protocols.

Troubleshooting



Before start

This protocol has three sections:

- **Section 1:** Setting up NCBI accounts (for new users)
- Section 2: Single-step data submission to SRA for raw reads and associated sequence metadata and to BioSample for sample metadata
- **Section 3**: Detailed steps for creating a BioProject (usually done once during the account set-up)

Associated protocols:

- Guidance for populating GenomeTrakr metadata templates (BioSample and SRA)
- NCBI Data Curation protocol for making updates, corrections, or retractions to your data.



Establish submission environmnet at NCBI

- 1 Set up a new NCBI submission environment for your lab:
 - 1.1: Create an NCBI user account
 - 1.2: Set up an NCBI submission user group for your lab
 - **1.4**: Bookmark the link to your submission portal
 - **1.5**. Identify or establish new BioProjects (detailed in **Step 3**)

Ready for data submission:

After these steps are complete you can proceed with data submission in Step 2.

1.1 Create an NCBI user account at NCBI: https://www.ncbi.nlm.nih.gov/account. This will be your own individual user account at NCBI.





***NCBI login changes updated in June, 2021. Read more here.

1.2 Establish an NCBI submission user group for your laboratory.

We recommend using this user group for all NCBI submissions related to microbial genome surveillance. This will link your laboratory's NCBI data ownership to the user group and not to individuals, allowing anyone in the current group to perform updates or retractions and answer inquiries from the NCBI staff, even if there's been a complete turnover of staff since the original data submission.

User groups also ensure consistent data ownership across BioProjects, BioSamples, and sequence data. If your laboratory has non-overlapping research groups submitting and managing data at NCBI, multiple user groups can be established to track these efforts separately.

Your laboratory might already have a submission group established! Check the "Group" tab in the submission portal, https://submit.ncbi.nlm.nih.gov/groups/. Ask your colleagues to do the same thing, to ensure your laboratory doesn't already have one in place.

Submission Portal

Groups

Group Id	Full name	Aliases
fda	FDA Center for Food Safety and Applied Nutrition	FDA/CFSAN
fda_ny	FDA/CFSAN/NY_State	
fda_mdh	FDA/CFSAN/MDH	

Creating a new submission group:

1. Submit an email request to **submit-help@ncbi.nlm.nih.gov** containing the following information:



Note

Dear NCBI help staff,

Please establish a new user group for my laboratory. I'm including the following information to help set up the group:

Short name of the group (abbreviation, e.g. "fda_ny")

Full name of the group (e.g. "NY Wadsworth microbial pathogen submission group")

Contact email(s) to start the group

Institution and department or group

Physical address including country

Primary contact person, first and last name plus email.

* if you have existing submissions you want to be converted, please request the ownership change in this email.

i.e., Please assign this new user group to the following BioProjects and linked data.

Thank you

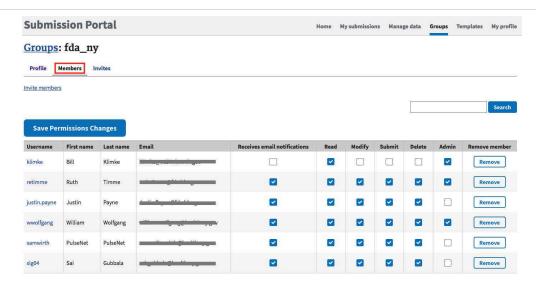
2. Look for an email reply entitled "NCBI Submission Portal Group invitation" and click on the enclosed link to accept the invitation.

1.3 Managing your NCBI submission user group.

After a user group has been established it can be edited for membership and permissions by clicking in the "group" tab of the submission portal (https://submit.ncbi.nlm.nih.gov/groups/), then on the Group Id hyperlink, e.g 'fda_ny' in the above example.

Users with admin privileges can update contact information in the "profile" tab and membership in the "Members" tab. New members can be invited by clicking on the "Invite members" link.





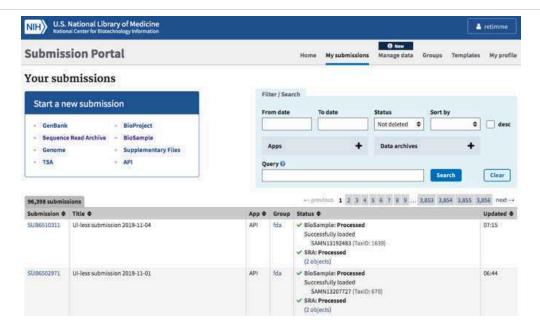
This user group should be kept up-to-date as members enter and leave the laboratory.

Permissions levels:

- READ: primarily for collaborators who can see the submissions, but not edit them.
- MODIFY, SUBMIT, DELETE: Permissions to submit, modify, or retract data (members usually have all or none of these permissions)
- ADMIN: Can invite or remove members of the submission group. Ensure that at least one (or more) members of your group have ADMIN privileges.
- 1.4 **Bookmark "my submissions"** at NCBI: https://submit.ncbi.nlm.nih.gov/subs/. This is the page where you view and track all of your past submissions.

If you see a blank page with a yellow box in the upper right corner saying "please login", click this link and login using the credentials created in **Step 1.1**.





1.5 Identify or establish new BioProjects (Umbrella and/or Data BioProjects)

Umbrella BioProjects. If you are already part of a surveillance network, (e.g. GenomeTrakr, NARMS, Vet-LIRN, or PulseNet) you will use one of their established umbrella BioProjects. For reference, here is a list of the major Umbrella projects for GenomeTrakr and Vet-LIRN.

GenomeTrakr Umbrella projects

(https://www.ncbi.nlm.nih.gov/bioproject/PRJNA593772):

Salmonella enterica	PRJNA183844
Listeria monocytogenes	PRJNA514048
Escherichia coli	PRJNA230919
 Vibrio parahaemolyticus 	PRJNA245885
Campylobacter sp.	PRJNA258021
Clostridium botulinum	PRJNA290488

Vet-LIRN Umbrella projects:

	Salmonella enterica	PRJNA314607
•	Escherichia coli and Shigella	PRJNA316449
	Staphylococcus	PRJNA316451

If you need to establish a new umbrella BioProject, follow instructions in **Step 3** with modifications for creating a new Umbrella BioProject (**Step 3.12**).

Data BioProjects. Does your laboratory have an established data BioProject for this effort? If not please follow the instructions in **Step 3** for creating a new one.



Note

More information:

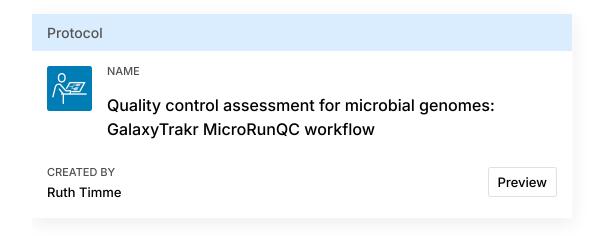
Learn more about data vs umbrella BioProjects in Step 3

Data submission (BioSample and SRA)

2 Data submission (source metadata and sequence data):

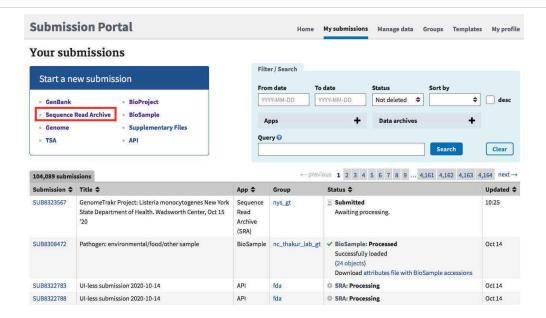
This protocol follows a one-step data submission process where the source metadata is submitted through the same submission workflow as the sequence data.

**Before submission, ensure that your sequences meet the quality control (QC) thresholds for your surveillance network. You can follow your own internal QC process or use FDA's free GalaxyTrakr platform:



Navigate to the My Submissions page in the NCBI Submission Portal: https://submit.ncbi.nlm.nih.gov/subs/

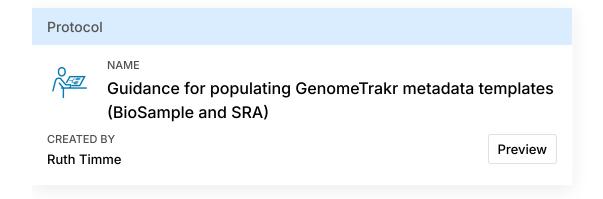




Click "Sequence Read Archive" to start a submission.

2.1 Download and populate the sample (BioSample) and sequence (SRA) metadata templates:

Custom metadata templates and guidance are available in the following protocol:

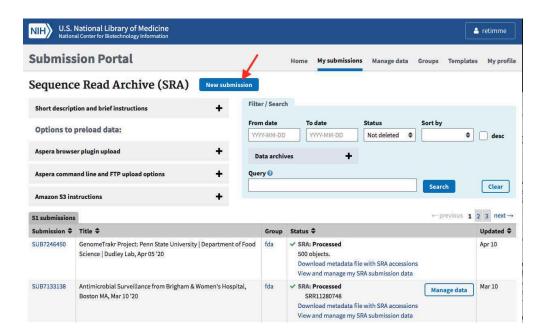


Note

Organize your submissions by BioProject, only submitting to a single BioProject per submission workflow. Populate the metadata spreadsheets for each isolate you intend to submit (you can submit metadata for a single isolate or a collection of isolates under a single BioProject).



2.2 Click the "New submission" box.

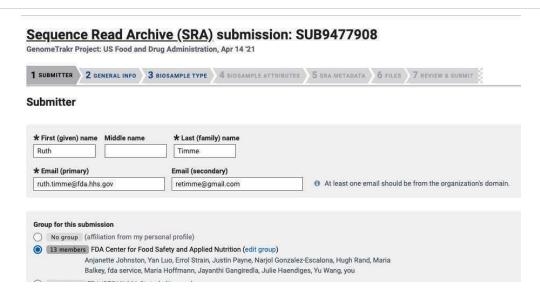


2.3 SUBMITTER tab:

Populate with submitter info. The "submitter" is the name of the person, or user group, who is physically doing the submissions, not a supervisor or PI.

Select the appropriate submission group name (see **Step 1.2** for creating a new submission group), and describe the submitting organization or laboratory name. This will be auto-populated from the contact info you included in your NCBI user account. Click "Continue" to proceed.





2.4 **GENERAL INFO tab:**

1. BioProject: Did you already have a data BioProject for this effort? If not please follow instructions in Step 3 for creating a new data or umbrella BioProject. Return back to this sub-step with the data BioProject accession in hand.

Click "Yes" and paste in your data BioProject accession, e.g. PRJNA614995.



Sequence Read Archive (SRA) submission: SUB9477908 GenomeTrakr Project: US Food and Drug Administration, Apr 14 '21 1 SUBMITTER 2 GENERAL INFO 3 BIOSAMPLE TYPE 4 BIOSAMPLE ATTRIBUTES 5 SRA METADATA 6 FILES 7 REVIEW & SUBMIT **General Information BioProject** BioProject describes the goal of your research effort. \bigstar Did you already register a BioProject for this research, e.g. for the submission of the reads to SRA and/or of the genome to GenBank? O Yes No * Existing BioProject PRJNA186035 PRJNA186035: GenomeTrakr Project: US Food and Drug Administration BioSample 1 The BioSample records the detailed biological and physical properties of the sample that was sequenced. A BioSample can be used in more than one BioProject since it should be used for all the data that were obtained from that sample. Usually SRA data sets are generated from more than one \bigstar Did you already register a BioSample for this sample, e.g. for the submission of the reads to SRA and/or of the genome to GenBank? Yes No Release date 0 Note: Release of BioProject or BioSample is also triggered by the release of linked data. * When should this submission be released to the public? Release immediately following processing Release on specified date or upon publication, whichever is first Please allow 24-48 hours for propagation of the data to the NCBI SRA public site.

- 2. BioSample: Click "NO" here. You will be registering BioSamples within this current submission
- 3. Release date: Choose "Release immediately following processing".
- 4. Click Continue.

Continue

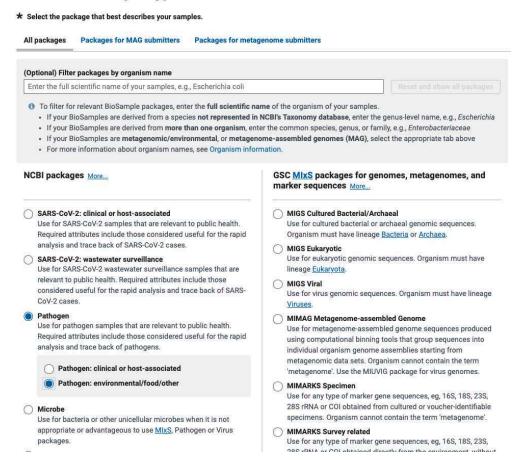
2.5 **BIOSAMPLE TYPE tab:**

You are choosing the appropriate metadata package here for your sample (i.e. what kind of samples are you submitting?).



Select "Pathogen", then "Pathogen:environmental/food/other" for microbial pathogen submissions.

Preview BioSample Types and Attributes



2.6 **BIOSAMPLE ATTRIBUTES** tab:

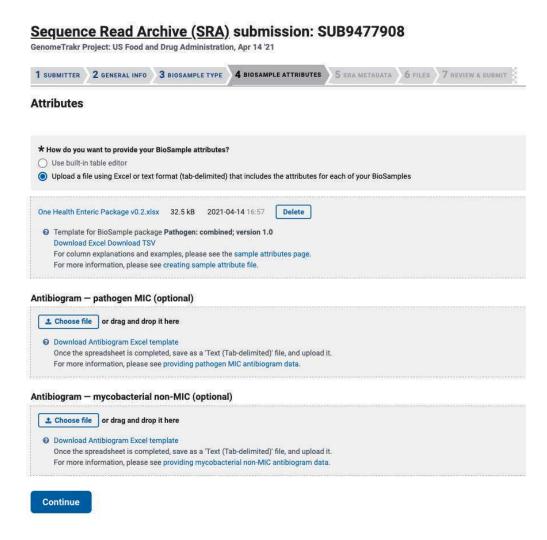
Choose "Upload a file using Excel or text format (tab-delimited) that includes the attributes for each of your BioSamples".

Then click "Choose File" and browse to your populated metadata template.



Note

If you have not yet populated your GenomeTrakr BioSample metadata template, see Step 2.1.



Antibiogram data: please provide if you have it!

Click "Continue".

NCBI will do a validation check on your metadata. Resolve any red "errors" reported back by editing the spreadsheet and replacing the uploaded file. Review any yellow "Warnings" and proceed if everything looks ok.



Click "Continue".

2.7 **SRA METADATA tab:**

Choose: "Upload a file using Excel or text format (tab-delimited)".

Sequence Read Archive (SRA) submission: SUB9477908 GenomeTrakr Project: US Food and Drug Administration, Apr 14 '21 1 SUBMITTER 2 GENERAL INFO 3 BIOSAMPLE TYPE 4 BIOSAMPLE ATTRIBUTES 5 SRA METADATA 6 FILES 7 REVIEW & SUBMIT SRA metadata For more detailed help with SRA submission please read the SRA Submission Wizard Help. ★ How do you want to provide your metadata? Use built-in table editor Upload a file using Excel or text format (tab-delimited) * Metadata file SRA_metadata-GTexample.xlsx 53.7 kB 2021-04-14 17:11 Delete Use one of these ways to fill out SRA metadata: Download Excel spreadsheet (designed to make it easier to select the correct metadata values), edit. save and then upload the modified Excel file. Continue

Upload your populated **SRA metadata template** (see Step 2.1 for where to get this file)

Click "Continue".

NCBI will do a validation check on your sequence metadata. Resolve any red "errors" reported back by editing the spreadsheet and replacing the uploaded file. Review any yellow "Warnings" and proceed if everything looks ok.

Click "Continue".

2.8 Files tab:



Each laboratory will establish its own path for transferring files.

In general, selecting the web browser option should work for uploading ~48 sequences at a time. For a more stable internet connection, your laboratory can use FTP or Aspera. Directions for doing so pop up after clicking the FTP radio button.

- Each file must be listed in the <u>SRA metadata table you uploaded</u>. If you are uploading a **tar** archive, list each file name, not the archive name.
 - Unique file names that do not contain any sensitive information should be used for all files. File
 names as submitted appear publicly when data is retrieved from the cloud.
 - Files can be compressed using gzip or bzip2, and may be submitted in a tar archive, but archiving or compressing your files is not required. Do not use zip!

* How do you want to provide files for this submission?

- Web browser upload via HTTP or Aspera Connect plugin

 Do not use web browser HTTP upload if you are uploading files over 10 GB or more than 300 files.
- FTP or Aspera Command Line file preload
 All files for a submission must be uploaded into a single folder.
- Amazon S3 bucket

Note

It is generally not recommended to check the Auto-submission box as this would not allow you to edit corrections if needed.

2.9 **REVIEW & SUBMIT tab:**

Check over your entire submission, then click submit.

If corrections are needed, you can go back and select individual tabs to edit your submission.

Note

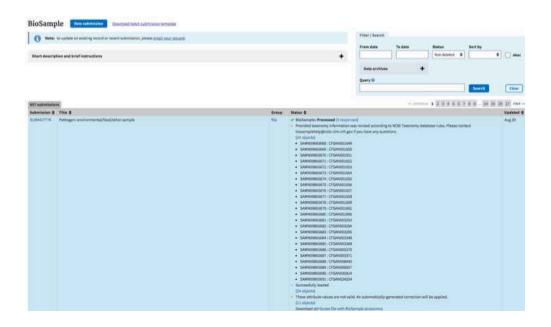
If you are having trouble finalizing your submission, contact the relevant NCBI database for assistance and include your submission ID in the email subject (SUB######):

BioSample (for source metadata issues): biosamplehelp@ncbi.nlm.nih.gov **SRA** (for raw sequence or sequence metadata issues): sra@ncbi.nlm.nih.gov



2.10 **BioSample accessions:**

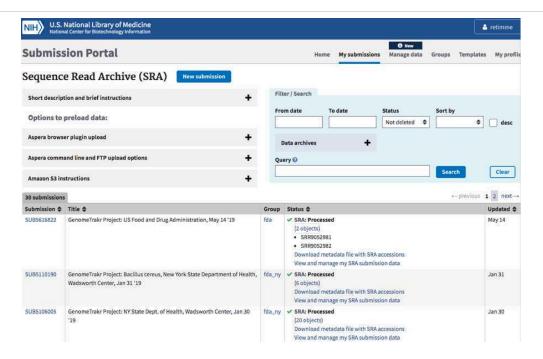
BioSample accessions will be automatically created upon submission and will be available on the "my submissions" page of the Submission portal by clicking on "## objects" within the submission record. You can also download by clicking the "Download attributes file with BioSample accessions". Accessions will start with SAMNxxxxxxxx. You will also receive an email within 12 hours, but typically much faster, containing these same accessions.



2.11 **SRA Accessions:**

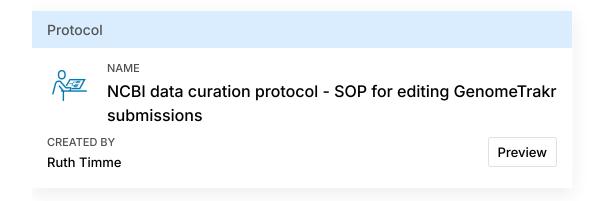
SRA run accessions will be available on the "my submissions" page of the Submission portal by clicking on "## objects" within the submission record. You can also download by clicking the "Download metadata file with SRA accession". Accessions will start with SRRxxxxxxx." You will also receive an email with these same accessions within 24 hours, but typically much faster, containing these same accessions.





2.12 Important data stewardship and curation notes:

- Develop an internal melDthod for storing and tracking your BioSample and SRR accessions! They are required for making future updates to your records.
- For updates, corrections, or retractions to your BioSample and SRA records, follow the guidance provided in the NCBI Curation Protocol. Some edits can be made within the submission portal and others need to be done via email.





Safety information

Caution: It is possible for a single BioSample to have more than one SRR ID. Two scenarios include:

- 1. Two runs were submitted for the same isolate/BioSample, which is not generally recommended for surveillance. Follow **Step 3** in the NCBI curation protocol to retract one of them).
- 2. if the initial submission was retracted and new a new run was submitted. It's important to keep track of both IDs, even if one was retracted.

BioProject Creation

3 Create a new BioProject

BioProjects are an organizing tool at NCBI that pulls together different kinds of data submitted across multiple NCBI databases. Each BioProject has a unique URL, providing a home page with a title, description, links to lab websites, publications, funding resources associated with a particular project, along with links to the deposited data. A basic **data BioProject** holds actual sequence data, assemblies, and their associated metadata. An **umbrella BioProject** is a way to group two or more data BioProjects together, which is useful for coordinating disease surveillance and for looking across the grouped BioProjects in a single view.

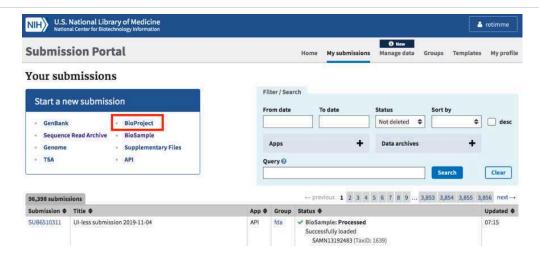
This protocol describes the steps for creating a new data BioProject linked to an existing umbrella BioProject (usually established by a coordinating group, e.g. GenomeTrakr, NARMS, Vet-LIRN).

*If you need to create a new Umbrella BioProject, modifications are summarized in Step **3.12.**

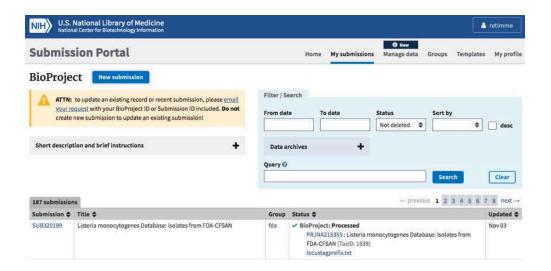
3.1

Navigate to the "My Submissions" page, https://submit.ncbi.nlm.nih.gov/subs/, and click "BioProject" in the "Start a new submission" box.





3.2 Click the "New submission" box:



3.3 Submitter tab:

Populate with submitter info. An NCBI "submitter" is the name of the person or submission group who is managing the submissions, not a supervisor or PI.

Select the appropriate submission group name (see **Step 1.2** for creating a new submission group), and describe the submitting organization or laboratory name. This will be auto-populated from the contact info you included in your NCBI user account.

3.4 **Project type tab:**



Project data type: Genome sequencing and assembly.

Sample scope:

For a Data BioProject: select multi-isolate. This will allow you to submit multiple isolates or strains of the same species.

3.5 Target tab:

For a Data BioProject: Populate ONLY the Organism name here, usually Genus species, or just Genus if your laboratory does not determine species, e.g., Salmonella enterica. Leave the strain info and Description fields blank.

Leave the strain info and Description fields blank.

3.6 General info tab:

Click "Release immediately following processing".

Include a brief title describing the effort.

 Data BioProject Title: e.g., "GenomeTrakr Project: NY State Dept. of Health, Wadsworth Center".

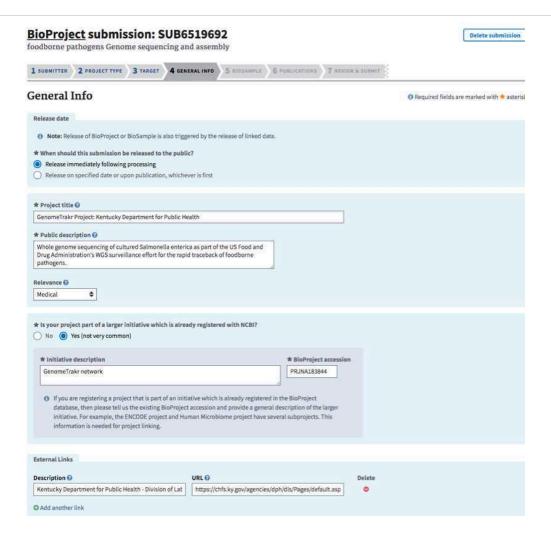
Public Description: e.g., "Whole-genome sequencing of pure-cultured microbial pathogens as part of XXXX surveillance effort."

Relevance: medical.

Is your project part of a larger initiative that is already registered at NCBI?

■ Data BioProjects. Click "Yes" and include a brief description and umbrella BioProject accession number (see Step 1.5). This will properly link your data project to the umbrella.





3.7 BioSample tab:

Leave blank!! You will create biosamples separately.

3.8 Publications tab:

If relevant, include publications from your laboratory.

3.9 Review and Submit tab:

Check if everything looks correct and edit if necessary, then click "submit."

3.10 The BioProject accession will be available within a few minutes on the "my submissions" page of the Submission portal, "PRJNAxxxxxx." You will also receive an email containing the new accession.





3.11 If you are part of a coordinated surveillance effort, like GenomeTrakr, please alert the coordinating body that a new BioProject was created under an existing umbrella.

For GenomeTrakr, contact genomeTrakr@fda.hhs.gov

3.12 Creating a new Umbrella BioProject:

Proceed as outlined in the above steps with the following modifications:

PROJECT TYPE tab:

For an *Umbrella BioProject*: select multi-species. This will allow you to link multiple data BioProjects representing different species under a single umbrella.

TARGET tab:

For an *Umbrella BioProject*: Leave the Organism name field blank. Include a list or description of species you intend to include in this effort. E.g. "bacterial foodborne pathogens", or "SARS-Cov-2"

GENERAL INFO tab:

Umbrella BioProject Title: e.g. "Microbial pathogen surveillance at NY State Dept. of Health, Wadsworth Center."

Is your project part of a larger initiative that is already registered at NCBI?

■ For an Umbrella BioProject: click "NO"



The last step is to email **bioprojecthelp@ncbi.nlm.nih**:

Example email:

Note

"Dear BioProject and PD help teams,

Please convert the PRJNA#### to an Umbrella BioProject. Our laboratory will be submitting data under the XXX effort (SARS-CoV-2, GenomeTrakr, Vet-LIRN, NARMS, HAI, or more general pathogen surveillance).

I'd be happy to provide any additional details you might need.

Thank you, "

After the conversion is complete you can use the new Umbrella accession to properly link any new data BioProjects being created.

3.13 **Important data stewardship and curation notes:**

- Develop an internal method for storing and tracking your BioProject accessions! They are required for every BioSample and sequence data submission to ensure proper linkage.
- Bookmark URLs for each of your data BioProjects to monitor the public-facing view of your submissions.
 - e.g. Virginia DCLS's GenomeTrakr Salmonella BP:

https://www.ncbi.nlm.nih.gov/bioproject/219491

• For updates to your BioProjects, follow the guidance provided in the NCBI Curation Protocol. Some edits can be made within the submission portal and others need to be done via email.



Protocol



NCBI data curation protocol - SOP for editing GenomeTrakr submissions

Preview

CREATED BY

Ruth Timme

February 16, 2023