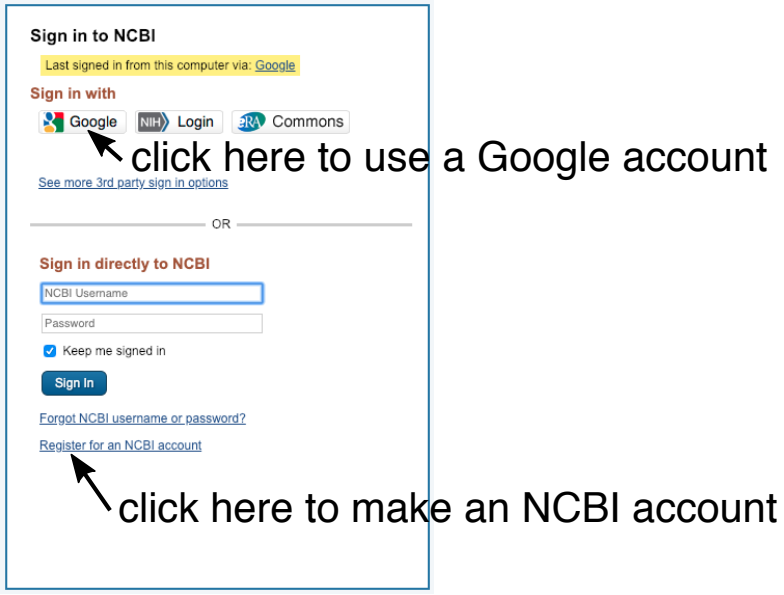
Molecular Microbiology 2022

Bioinformatics Protocol 11

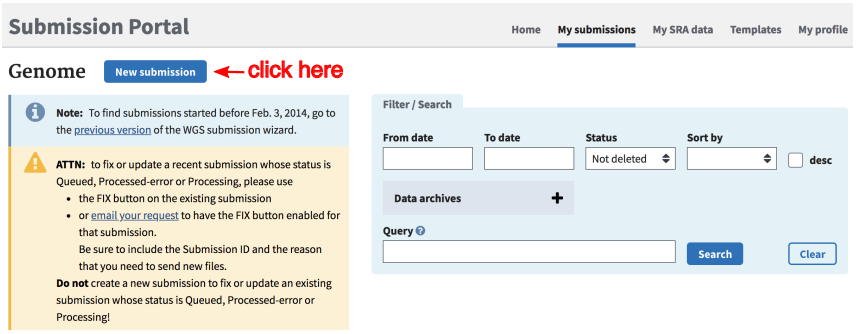
Submitting assembled genomes to NCBI

This protocol covers the submission of assembled prokaryotic genomes to NCBI as a Whole-Genome Shotgun (WGS) project.

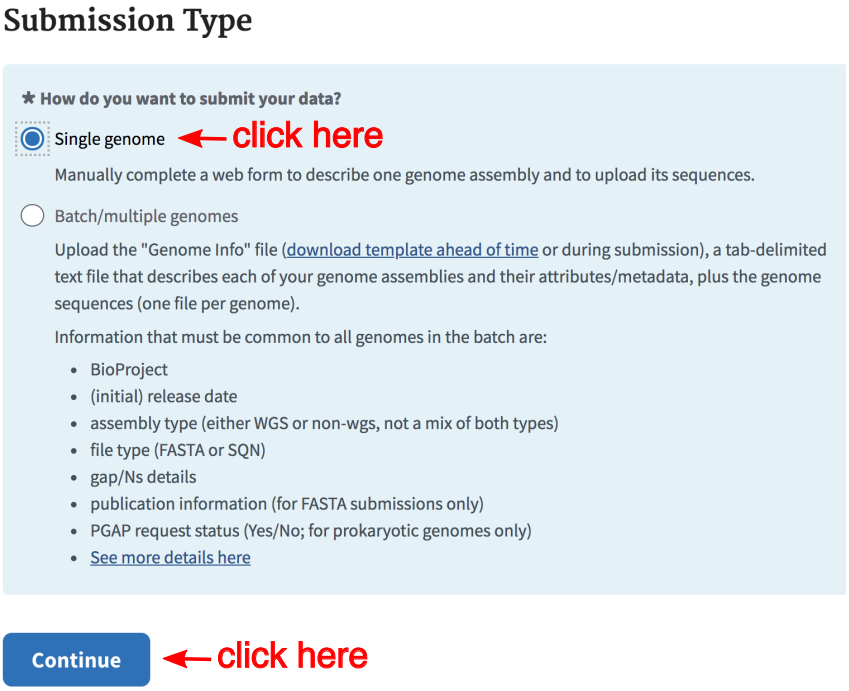
1. If you haven't already done so, download the `fasta` file containing your decontaminated scaffolds (or scaffolds from your raw spades assembly if no decontamination was necessary) to your own computer. The `.fna` file from your prokka annotation directory is the same thing.
2. Open the `fasta` file in Sublime Text and check for any scaffolds less than 200 bp in length (the `length_X` in each scaffold's name will tell you its length). Delete any scaffolds shorter than 200 bp and save the file.
3. Open a web browser and go to https://submit.ncbi.nlm.nih.gov/subs/genome/. Click *Login*. If you have a Google account you can log in using that, otherwise you will need to register for an NCBI account.



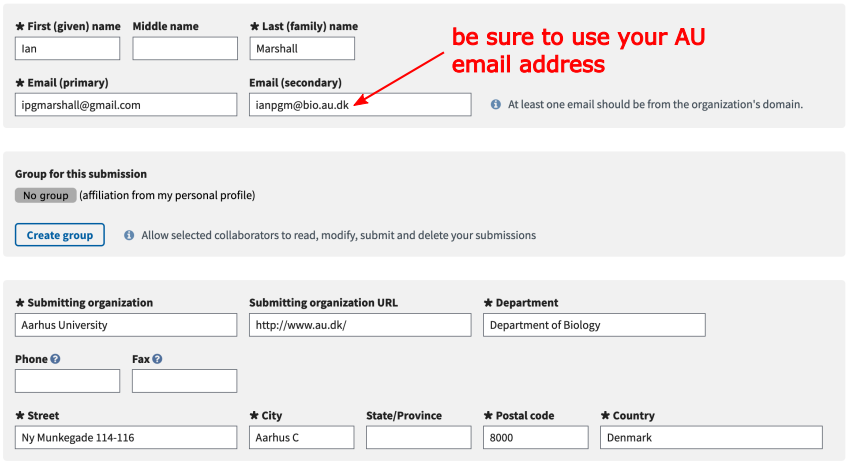
4. Click *New submission*.



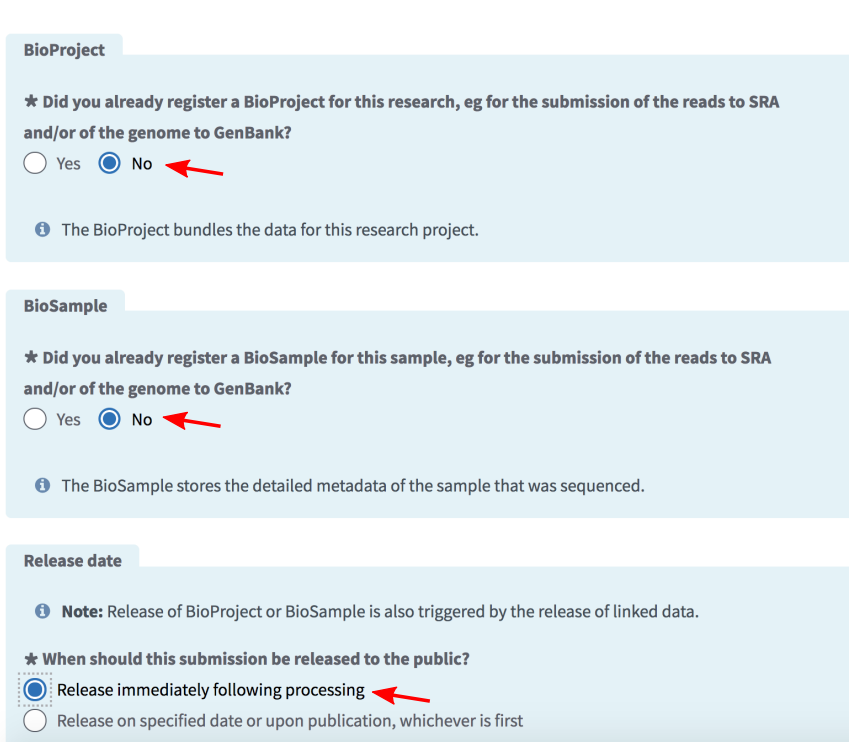
5. Select *Single Genome* and click *Continue*.



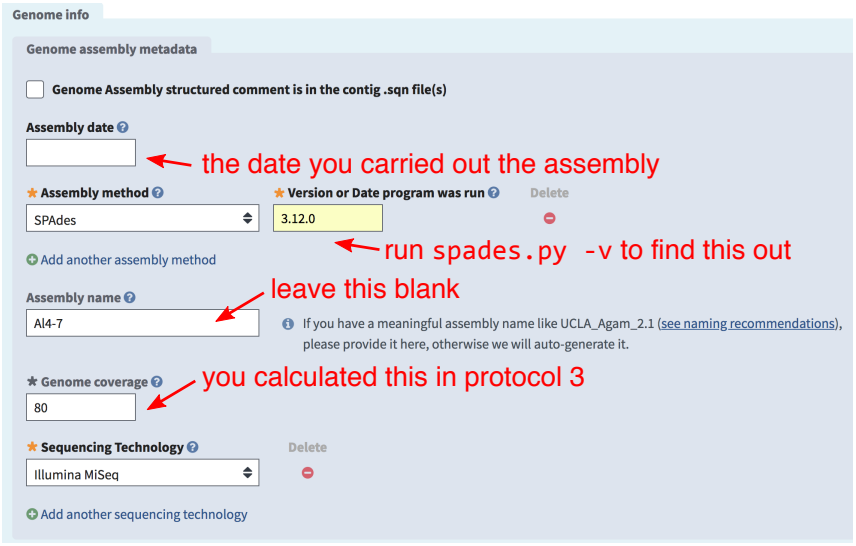
6. Enter your personal contact details, AU Biology, and click *Continue*. You need to include an AU email address in at least one of these email fields.



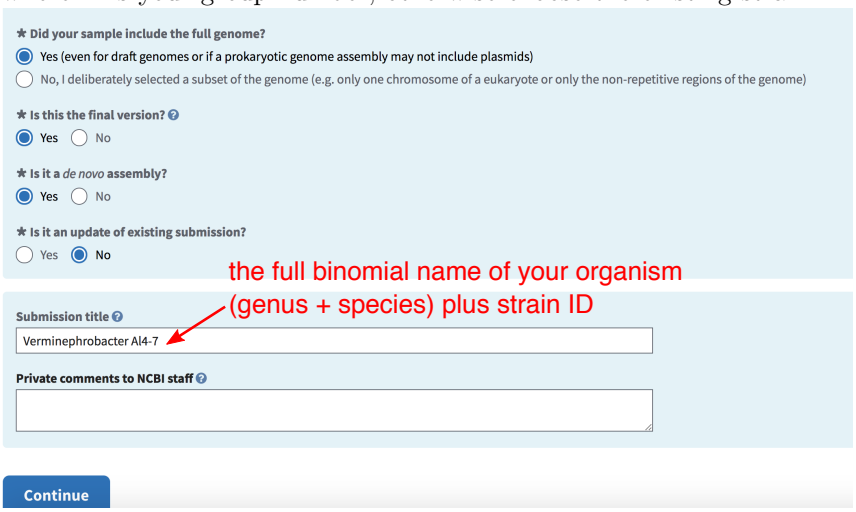
7. Select the following options then continue.



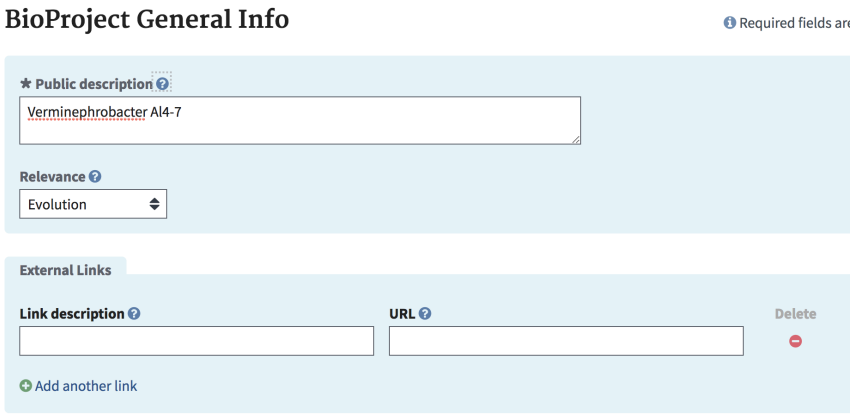
8. Enter information about your Genome Assembly.



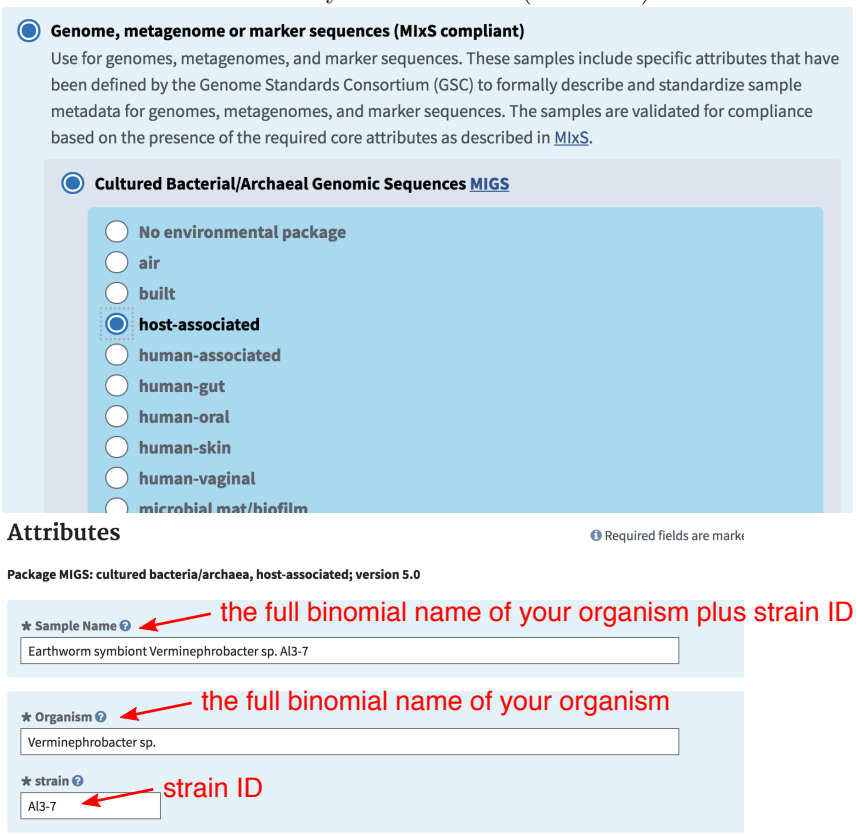
9. Tell NCBI that this is a full genome, and name your submission with the full binomial name for your species followed by strain ID (e.g. "Pseudomonas stutzeri IC-126"), then click continue. Groups using their own strains from Microbial Physiology use a strain ID "MM2021_X" where X is your group number, otherwise choose the existing strain ID.

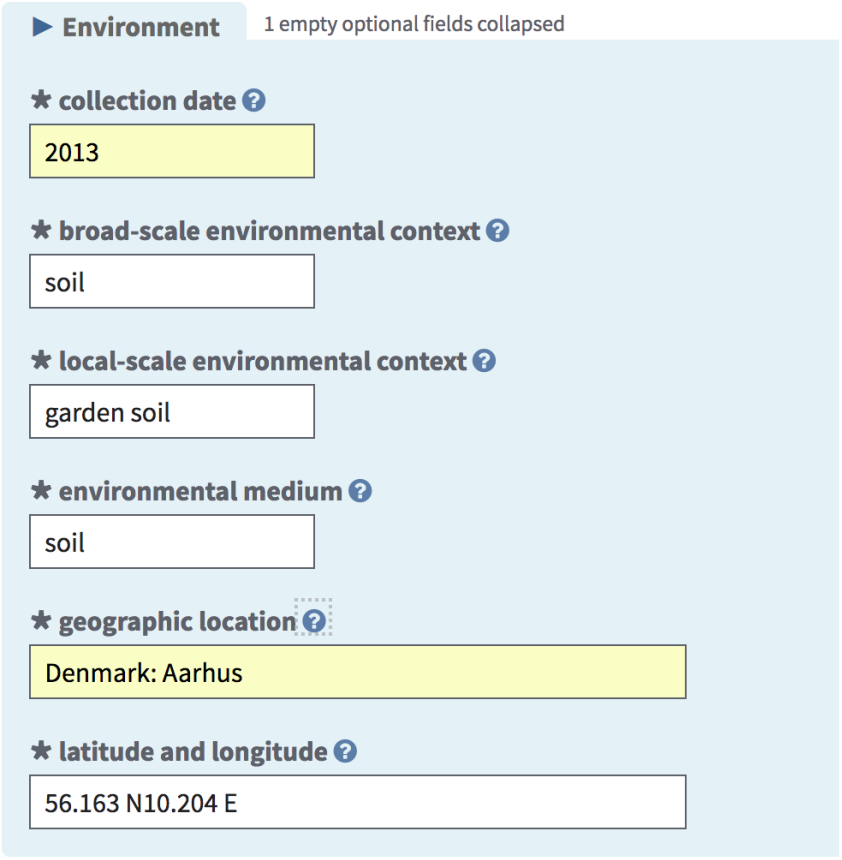


10. Give the organism a public name the same as your submission name, and select what "relevance" your study has from the drop-down menu. You should decide on the relevance based on the background of your organism.

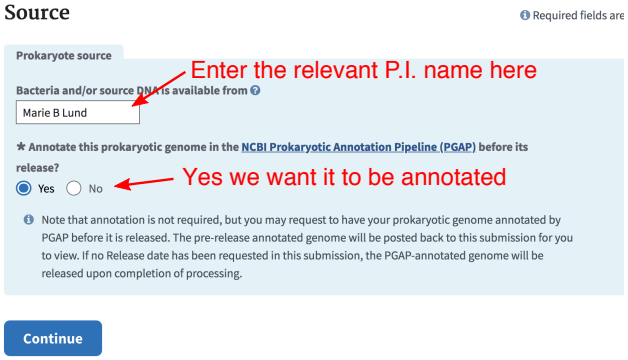


11. Select an appropriate submission package for your genome depending on the kind of sample it was isolated from. This will determine the kind of metadata to be included in your submission (see below).





12. After adding the relevant metadata, you need to specify where the strain is available (enter the name of the relevant P.I., i.e. Kai Finster) and specify the yes, we would like to have the genome annotated.



13. Now select some final options and (finally!) upload your `fasta` file from step 2. Be sure to have the instructor check your submission details before you upload!

Which of these 3 options describes this genome submission?

- ( ) 1. Each chromosome is in a single sequence and there are no extra sequences
  - There can still be gaps within the sequences.
    We will prompt you to provide the information for any Ns that represent gaps.
  - Internal sequences must be arranged in the correct order and orientation.
    Sequences concatenated in unknown order are not allowed.
  - Plasmids and organelles can still be in multiple pieces.
  - If the sequences are assembled using an AGP file, choose the next option.
- (•) 2. One or more chromosomes are still in multiple pieces and/or some sequences are not assembled into chromosomes
  - This will be processed as a WGS genome and may include AGP files in the submission
  - There can still be gaps within the sequences.
    We will prompt you to provide the information for any Ns that represent gaps.
  - Internal sequences must be arranged in the correct order and orientation.
    Sequences concatenated in unknown order are not allowed.
- ( ) 3. We are submitting just the AGP file(s) for a genome assembly; the components of the AGP file are already in GenBank

Select file type for the sequences

( ) ASN.1 (.sqn) (•) FASTA

Request preload folder

Select upload type

( ) I have all files preloaded for this submission
(•) I will upload all the files now via HTTP/Aspera

Current versions of browsers Firefox, Chrome, Safari or Internet Explorer are recommended.
To upload large eukaryotic files (larger than 2GB), please use Aspera Connect plugin.
Please note: in order to use Aspera for file upload with Chrome, you need to update Aspera Connect plugin to version 3.6 or newer. More details…

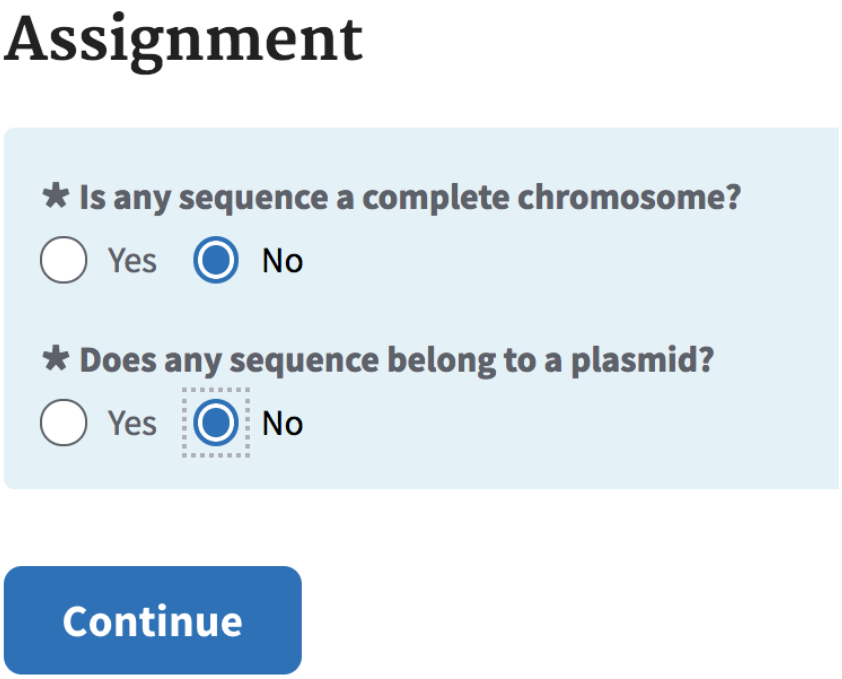
Upload FASTA

Choose Files No file chosen

Name	Size	Created	Delete
Al3-7.fasta	5.0 MB	2/27/2019 20:26	⛔

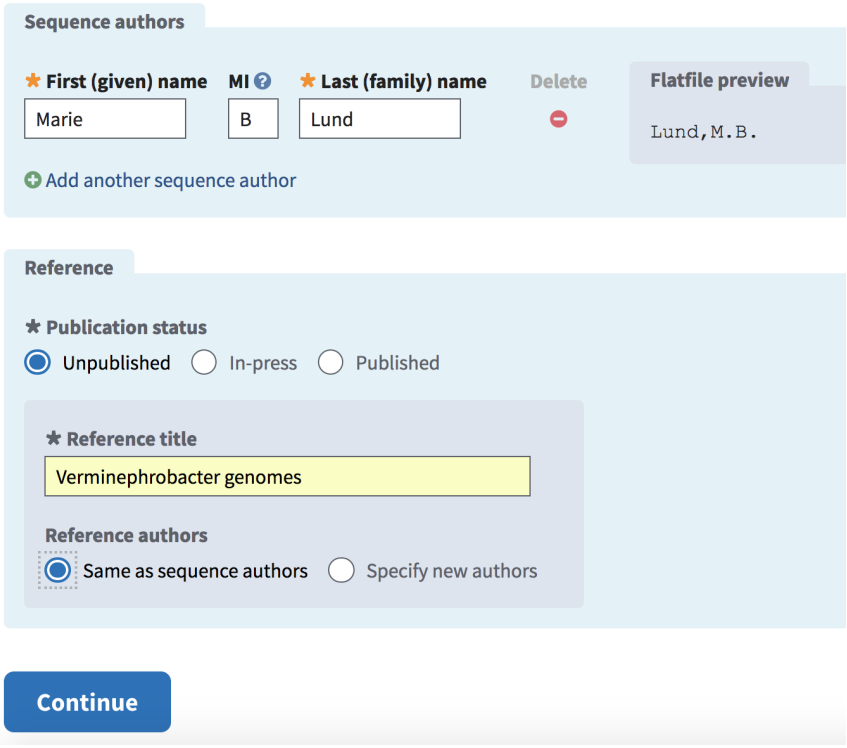
Do you have AGP files that assemble the individual contigs into scaffolds or chromosomes, OR assemble the submitted gapped sequences into chromosomes?

( ) Yes (•) No



14. Finally, specify the authors of your submission (your group members, Ian P.G. Marshall, and Kai Finster). Under *Reference title* since there's no publication associated with this, you should give it a name that makes since ("Draft genome sequence of Bacillus magnificus MM2021_2" or something along those lines).

References


Sequence authors
First (given) name
MI
Last (family) name
Delete
Flatfile preview
Marie
B
Lund
Lund,M.B.
Add another sequence author
Reference
Publication status
Unpublished
In-press
Published
Reference title
Verminephrobacter genomes
Reference authors
Same as sequence authors
Specify new authors
Continue