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1 Abstract/Introduction

Timely submission of viral sequence and corresponding contextual data by public health laboratories is an essential step to SARS-CoV-2 biosurveillance. This enables real-time updating of repositories of sequence data, hence real-time tracking of information on the virus as gathered from the sequence data and metadata as well. There are various sequence repositories or

Submission of sequence and contextual data to GISAID, INSDC repositories, or other databases

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phagesubgrantph

ABSTRACT

Timely submission of viral sequence and corresponding contextual data by public health laboratories is an essential step to SARS-CoV-2 biosurveillance. This enables real-time updating of repositories of sequence data, hence real-time tracking of information on the virus as gathered from the sequence data and metadata as well. There are various sequence repositories or databases (with a section dedicated to SARS-CoV-2 viral sequences) that are publicly-available online, such as GISAID and the INSDC repositories.

The previous project involved submission of the sequences generated by the PGC Mindanao workflow to the GISAID database. In collaboration with BugSeq on the PHA4GE subgrant, PGC Mindanao was also able to upload and submit the previouslygenerated SARS-CoV-2 sequences to NCBI, a database that is part of the INSDC, which in turn subsequently released these sequences to its public database successfully.

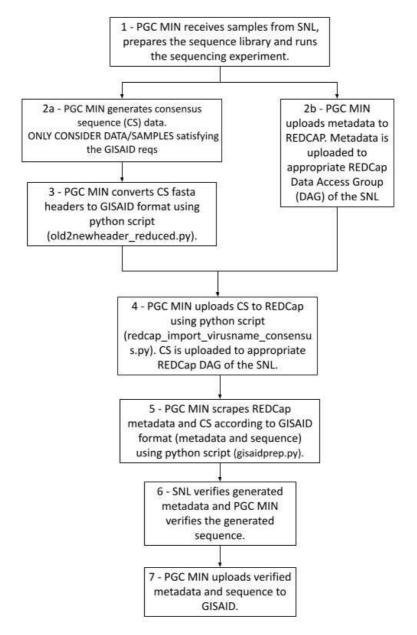
databases (with a section dedicated to SARS-CoV-2 viral sequences) that are publicly-available online, such as GISAID and the INSDC repositories.

The previous project involved submission of the sequences generated by the PGC Mindanao workflow (refer to protocol on "Introduction and Lineage Assignment of Assembled Sequences") to the GISAID database. In collaboration with BugSeq on the PHA4GE subgrant, PGC Mindanao was also able to upload and submit the previously-generated SARS-CoV-2 sequences to NCBI, a database that is part of the INSDC, which in turn subsequently released these sequences to its public database successfully.

The protocol below outlines the PGC Mindanao workflow for the submission of sequence and contextual data to GISAID (Section 2). A short section shows how the PHA4GE contextual data package was used in the submission process (Section 3). The process of submission of sequence and contextual data by PGC Mindanao to the NCBI database is also outlined (Section 4). A comparison of results from GISAID and NCBI submissions is shown (Section 5).

2 PGC Mindanao workflow

This section outlines the PGC Mindanao workflow for the submission of sequence and contextual data to a public database. Figure 1 shows an overview of the entire workflow. The workflow ultimately deposits the sequence and contextual data to the online public database GISAID.



Note: Original python scripts provided by Paco Polotan of RITM and modified by Bioinfo Division PGCMin

Figure 1. Overview of the PGC Mindanao workflow for submission of sequence and contextual data to the GISAID database

After assembling and generating the consensus sequences from the viral samples, the sequences are assessed and filtered for the number of ambiguous bases and presence of frameshift mutations and unexpected stop codons in the sequences as confirmed by the sequencing data and not from sequencing artifacts. Sequencing having less than 50% ambiguous bases and have frameshift mutations and unexpected stop codons that are confirmed by the sequencing data will be included in the GISAID submission and those otherwise were not included

(refer to Section 2.6 - "Nextclade workflow" of the protocol "Introduction and Lineage Assignment of Assembled Sequences" for details). The headers of the multi-sequence FASTA files were renamed conforming to GISAID requirements (Section 2.1). Parallel to this, sequence metadata were also uploaded to REDCap according to the appropriate data access group of each Sub-National Laboratory (SNL) (Section 2.2).

Sequences were uploaded to REDCap using the script provided by the previous project (succeeding steps were also performed with scripts provided by the previous project). Similar to the metadata, the sequence data were uploaded to REDCap according to the appropriate data access group of each SNL. Both sequence data and metadata were scraped from the REDCap database using the provided Python script (Section 2.2). PGC Mindanao verified the sequences and the corresponding metadata. When verification was done, the sequences and the metadata were uploaded to GISAID through their submission protocol (Section 2.3).

2.1 Formatting of sequence data to standard form:

Various scripts were created and provided by the previous project to format heading of FASTA file to standard form and other process. The provided custom script "old2newheader_reduced.py" converted the header/s of the sequence FASTA file to conform to GISAID convention (Figure 2).

```
### Additional process of the second process
```

Figure 2. Screenshots of two of the scripts used in the workflow, "old2newheader_reduced.py" and "redcap_import_virusname_consensus.py"

2.2 Data entry into, and data "scraping" from REDCap Database:

Sequence data and contextual data/metadata (refer to protocol "Establishing processes to capture standardized contextual data" for details) was entered into the REDCap database of the previous project. Figure 3 shows the landing page after entering the login into the REDCap database, which is based on the data access group entered from the last login. Individual sample sequence data and metadata entries may be uploaded manually through the webpage GUI (through the green button "Add new record" and manual entry in individual sub-entry pages). Figure 4 shows the case metadata sub-entry page. Figure 5 shows the analysis metadata sub-entry page. Another approach to uploading data is through the "Data Import Tool" (Figure 6). Sequence data may also be uploaded through the use of the provided custom script "redcap_import_virusname_consensus.py" (Figure 2).

Data can be "scraped", or collected, from the same database by using the provided custom script "gisaidprep.py", which outputs a spreadsheet that conforms to GISAID submission requirements (Figure 7).

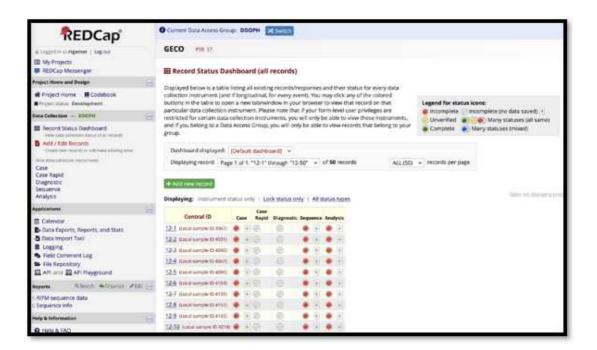


Figure 3. Main landing page after entering login of the REDCap database



Figure 4. Case metadata sub-entry page of a REDCap database sample entry

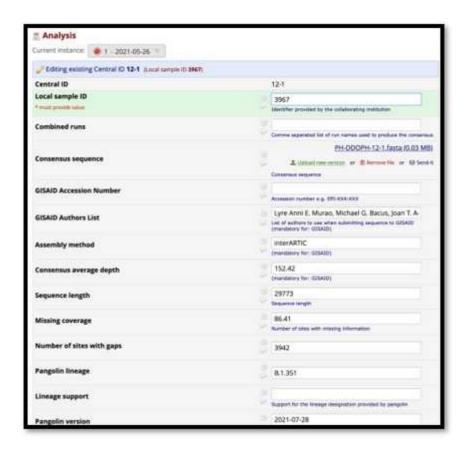


Figure 5. Analysis metadata sub-entry page of a REDCap database sample entry

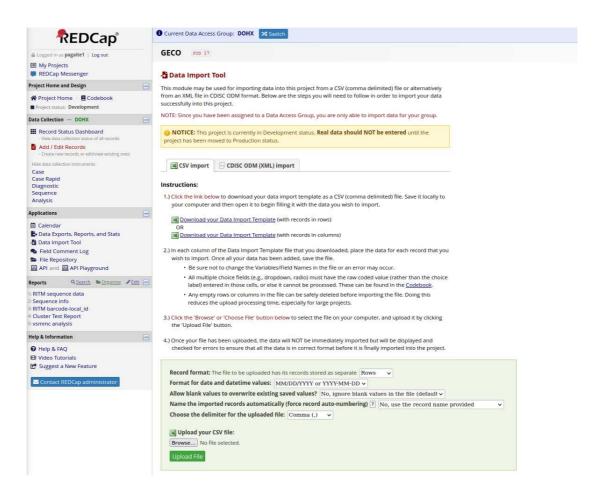


Figure 6. Data import tool of REDCap

2.3 Submission to GISAID database:

The spreadsheet outputted by "gisaidprep.py" (containing the sequence metadata) from the previous section, along with the final formatted sequence FASTA file, will be used for uploading the sequence data and metadata to GISAID. Figure 7 shows the spreadsheet outputted by "gisaidprep.py" metadata that will be submitted to GISAID in conjunction with the sequence data. Standard GISAID submission procedure was performed, wherein the recommended approach is through the use of the command-line tool CLI2. This process requires a live client ID for authentication, which may be requested by emailing the support team for this tool at GISAID (clisupport@gisaid.org). Figure 8 shows a number of the commands that can be issued by the CLI2 command-line tool.

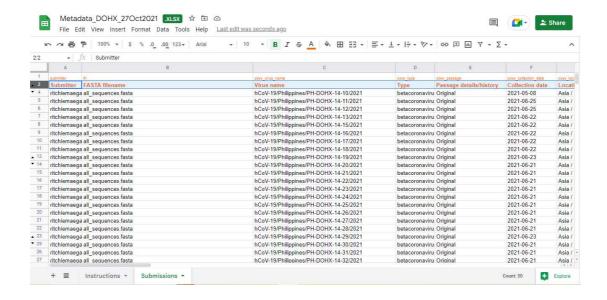


Figure 7. Spreadsheet outputted by "gisaidprep.py" script containing sequence metadata conforming to GISAID submission requirements

```
To get help on the upload sub-command, do cli2 upload -h:
   usage: cli2 upload [-h] [--database {EpiCoV,EpiFlu,EpiRSV}] [--token TOKEN] --metadata

→ METADATA --fasta FASTA

                       [--frameshift {catch_all,catch_novel,catch_none}] [--failed FAILED]
                           ← [--proxy PROXY] [--debug] [--log LOG]
   Perform upload of sequences and metadata to GISAID's curation zone.
   optional arguments:
                         show this help message and exit
   -h. --help
   --database {EpiCoV,EpiFlu,EpiRSV}
                         Target GISAID database. (default: EpiCoV)
   --token TOKEN
                         Authentication token. (default: ./gisaid.authtoken)
   --metadata METADATA The csv-formatted metadata file. (default: None)
                                               3
   -- fasta FASTA
                         The fasta-formatted nucleotide sequences file. (default: None)
   --frameshift {catch_all,catch_novel,catch_none}
15
                            'catch_none': catch none of the frameshifts and release
14
                               → immediately; 'catch_all': catch all frameshifts and
→ require email
                           confirmation; 'catch_novel': catch novel frameshifts and require
15
                               email confirmation, (default: catch_all)
   -- failed FAILED
                        Name of CSV output to contain failed records. (default: ./failed.
36
      ( out)
   --proxy PROXY
                        Proxy-configuration for HTTPS-Request in the form: http(s)://
17
       username:password@proxy:port. (default: None)
   --debug
                         Switch off debugging information (dev purposes only). (default:
18
       → True)
                         All output logged here. (default: ./upload.log)
   --log LOG
```

Figure 8. A number of the commands that can be issued by the CLI2 command-line tool

After submission of the sequence data and metadata to GISAID through the CLI2 tool, the data will be reviewed by curators at GISAID. The uploader will be notified by email of successfully-released samples (together with their corresponding individual accession IDs) from submit@gisaid.org, and the samples made immediately available to registered GISAID users. On the other hand, the uploader will be notified by email of unsuccessful sample/s from hcov-19@gisaid.org with the reason for the non-release of each of these sample/s. The uploader may resubmit with the corrected sequence/s or inform GISAID that the sequence/s is correct and is supported by the raw sequencing data.

3 PHA4GE contextual data package

The contextual data template spreadsheet from the package can be used to standardize submission to repositories. See protocol on "Establishing processes to capture standardized contextual data" for details.

4 NCBI GenBank submission process

BugSeq has conducted an orientation session on submission of sequence and contextual data to INSDC repositories, particularly NCBI GenBank. Sequences previously-generated by PGC Mindanao were submitted in actual during the orientation. This section outlines this process.

Figure 9 shows the landing page or submission portal for sequences in GenBank. The process is done entirely on webpage GUI at the online NCBI GenBank page. The page also shows the overview of previous sequence submissions.

Figure 10 shows the first step to submission, asking the uploader the type of sequences to be uploaded and other details (e.g. if it is SARS-CoV-2).

Figure 11 shows the page for the second step, which asks for details regarding the submitter.

Figure 12 shows the third step of the process, which asks for details regarding the sequencing technology used (e.g. sequencing platform and method).

Figure 13 shows the fourth step, which is the upload page for the sequence file and also asks when the sequences are to be released.

Figure 14 presents the fifth step, which is the sequence processing page and also where the uploader is given an option to automatically remove failed sequences.

Figure 15 shows the sixth step, which is the source information page and where the uploader is asked about the details on the sequence IDs.

Figures 16 and 17 show the seventh step of the process wherein source modifiers, or sample/sequence metadata, are provided. The uploader is given an option to provide the metadata by filling out the editable table within the page or uploading a tab-delimited table file containing the metadata. Figure 17 shows the editable table where the source modifiers are provided in the submission process in this case.

Figure 18 shows the eighth step asking details on references, such as sequence author information and status of the publication linked to the sequences.

Figure 19 shows the ninth step, which is the review of the sequence submission before uploading and submitting the sequence data and corresponding metadata to the NCBI GenBank database.

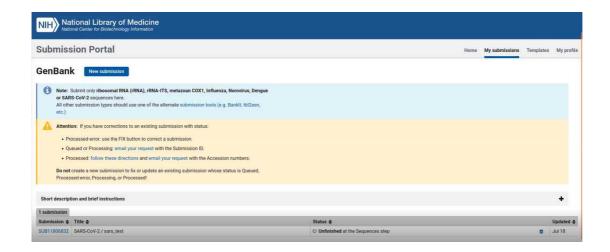


Figure 9. Landing page of the submission portal of NCBI GenBank

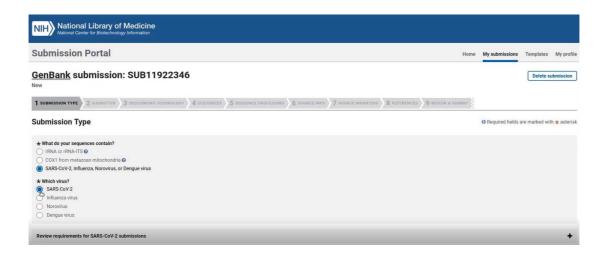


Figure 10. Submission type page

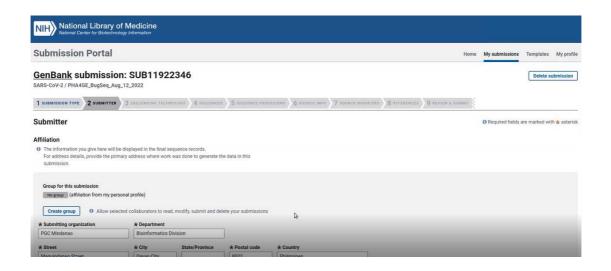


Figure 11. Submitter information page

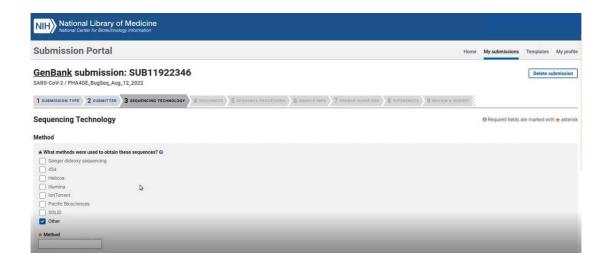


Figure 12. Sequencing technology page

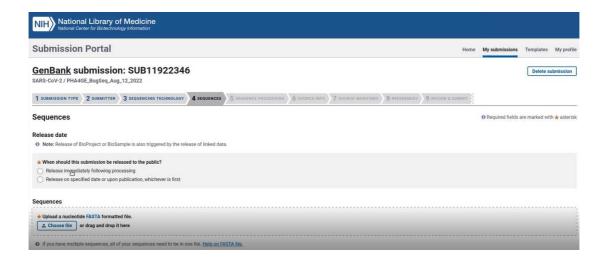


Figure 13. Sequence upload and information page

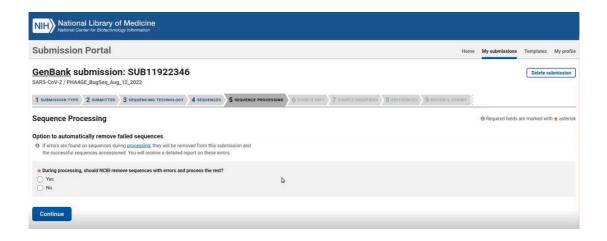


Figure 14. Sequence processing page

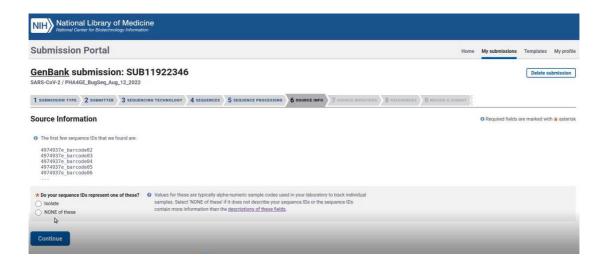


Figure 15. Source information page

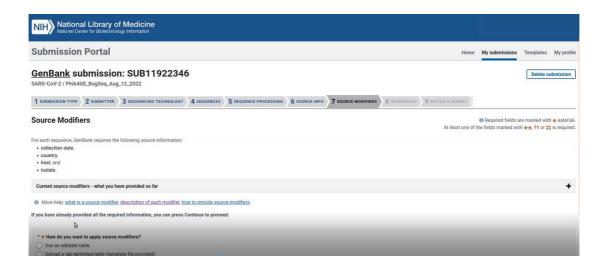


Figure 16. Source modifiers page

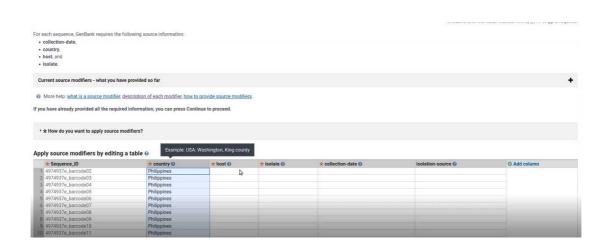


Figure 17. Editable table for inputting source modifiers

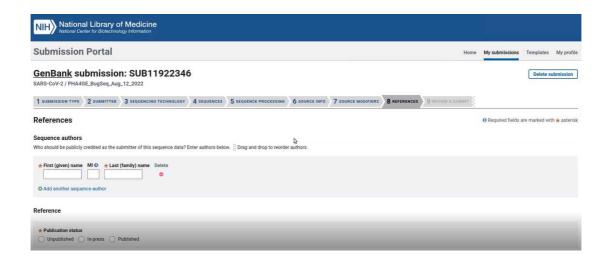


Figure 18. References page for the submission

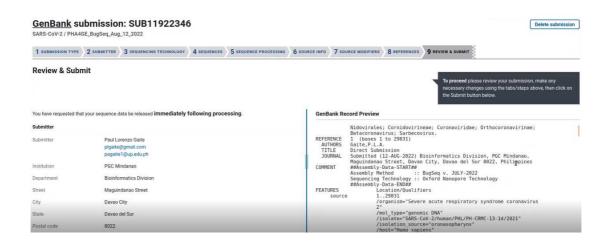


Figure 19. Review page for the submission

After submission of the sequence data and metadata to NCBI GenBank through its Submission Portal, the data will be reviewed by curators at NCBI GenBank. The uploader will be notified by email if the whole batch of sequences has been successfully-released or not, with the reason for the non-release of each unsuccessful sample. The uploader may resubmit with the corrected sequence/s through the Submission Portal or inform NCBI GenBank that the sequence/s is correct and is supported by the raw sequencing data.

5 Comparison of GISAID and GenBank submissions

Table 1 shows details of the sequences submitted to GISAID (from previous project) and GenBank (from this grant), such as if initial database submission was successful, step/s taken to

resolve initial unsuccessful submission, if resubmission was successful, and the corresponding accession ID given by the database (if applicable).

А	В	С	D	E	F	G	Н	I	J	K
Sample ID	GISA ID sub mitte d?	Initial GISAI D submi ssion succe ssful?	Resol ution if initial GISAI D submi ssion unsuc cessf ul	Resub missi on to GISAI D succe ssful?	GISAID accessio n ID	NCBI sub mitte d?	Initial NCBI subm ission succ essfu !?	Resol ution if initial NCBI submi ssion unsuc cessf ul	Resu bmis sion to NCBI succ essf ul?	NCBI accessi on ID
hCoV- 19/Philippine s/PH-CRMC- 13-14/2021	Yes	Yes	-	-	EPI_ISL_ 5934896	Yes	No	Corre cted seque nce	Yes	OP5224 26
hCoV- 19/Philippine s/PH-CRMC- 13-15/2021	Yes	Yes	-	-	EPI_ISL_ 5934897	Yes	Yes	-	-	OP5224 27
hCoV- 19/Philippine s/PH-CRMC- 13-16/2021	Yes	Yes	-	-	EPI_ISL_ 5934898	Yes	Yes	-	-	OP5224 28
hCoV- 19/Philippine s/PH-CRMC- 13-17/2021	Yes	Yes	-	-	EPI_ISL_ 5934899	Yes	Yes	-	-	OP5224 29
hCoV- 19/Philippine s/PH-CRMC- 13-18/2021	Yes	Yes	-	-	EPI_ISL_ 5934900	Yes	Yes	-	-	OP5224 30
hCoV- 19/Philippine s/PH-CRMC- 13-19/2021	Yes	Yes	-	-	EPI_ISL_ 5934901	Yes	Yes	-	-	OP5224 31
hCoV- 19/Philippine s/PH-CRMC- 13-20/2021	Yes	Yes	-	-	EPI_ISL_ 5934902	Yes	Yes	-	-	OP5224 32
hCoV- 19/Philippine s/PH-CRMC- 13-21/2021	Yes	Yes	-	-	EPI_ISL_ 5934903	Yes	Yes	-	-	OP5224 33
hCoV- 19/Philippine s/PH-CRMC- 13-22/2021	Yes	Yes	-	-	EPI_ISL_ 5934904	Yes	Yes	-	-	OP5224 34
hCoV- 19/Philippine s/PH-CRMC- 13-23/2021	Yes	No	Corre cted seque nce	Yes	EPI_ISL_ 5934905	Yes	No	Corre cted seque nce	No	-
hCoV- 19/Philippine s/PH-DDOPH- 12-1/2021	Yes	Yes	-	-	EPI_ISL_ 5934981	Yes	Yes	-	-	OP5224 35

A	В	С	D	E	F	G	Н	I	J	K
hCoV- 19/Philippine s/PH-DDOPH- 12-2/2021	Yes	Yes	-	-	EPI_ISL_ 5934985	Yes	Yes	-	-	OP5224 36
hCoV- 19/Philippine s/PH-DDOPH- 12-3/2021	Yes	Yes	-	-	EPI_ISL_ 5934986	Yes	Yes	-	-	OP5224 37
hCoV- 19/Philippine s/PH-DDOPH- 12-4/2021	Yes	Yes	-	-	EPI_ISL_ 5934987	Yes	Yes	-	-	OP5224 38
hCoV- 19/Philippine s/PH-DDOPH- 12-5/2021	Yes	Yes	-	-	EPI_ISL_ 5934988	Yes	Yes	-	-	OP5224 39
hCoV- 19/Philippine s/PH-DDOPH- 12-6/2021	Yes	Yes	-	-	EPI_ISL_ 5934989	Yes	Yes	-	-	OP5224 40
hCoV- 19/Philippine s/PH-DDOPH- 12-7/2021	Yes	Yes	-	-	EPI_ISL_ 5934990	Yes	Yes	-	-	OP5224 41
hCoV- 19/Philippine s/PH-DDOPH- 12-8/2021	Yes	Yes	-	-	EPI_ISL_ 5934991	Yes	No	Corre cted seque nce	Yes	OP5224 42
hCoV- 19/Philippine s/PH-DDOPH- 12-9/2021	Yes	Yes	-	-	EPI_ISL_ 5934992	Yes	Yes	-	-	OP5224 43
hCoV- 19/Philippine s/PH-DDOPH- 12-10/2021	Yes	Yes	-	-	EPI_ISL_ 5934982	Yes	Yes	-	-	OP5224 44
hCoV- 19/Philippine s/PH-DDOPH- 12-11/2021	Yes	Yes	-	-	EPI_ISL_ 5934983	Yes	Yes	-	-	OP5224 45
hCoV- 19/Philippine s/PH-DDOPH- 12-12/2021	Yes	Yes	-	-	EPI_ISL_ 5934984	Yes	Yes	-	-	OP5224 46

Table 1. Comparison of GISAID and NCBI submission details