INTRODUCTION TO **NGS** DATA

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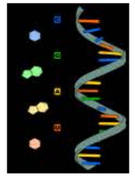
Today's Agenda

- Intro talk on NGS data formats
- Bioinformatics Module 1 (~30-40 min)
- Session wrap up & VM install guide

We have a sequence, now what?

How do we go from sample to actionable data?

Swab

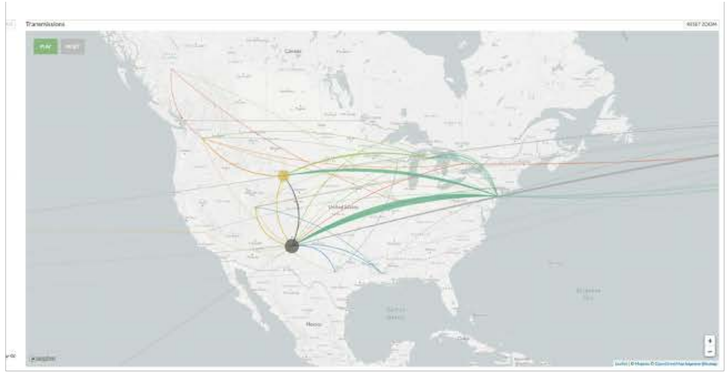


Isolate RNA

Sequencing

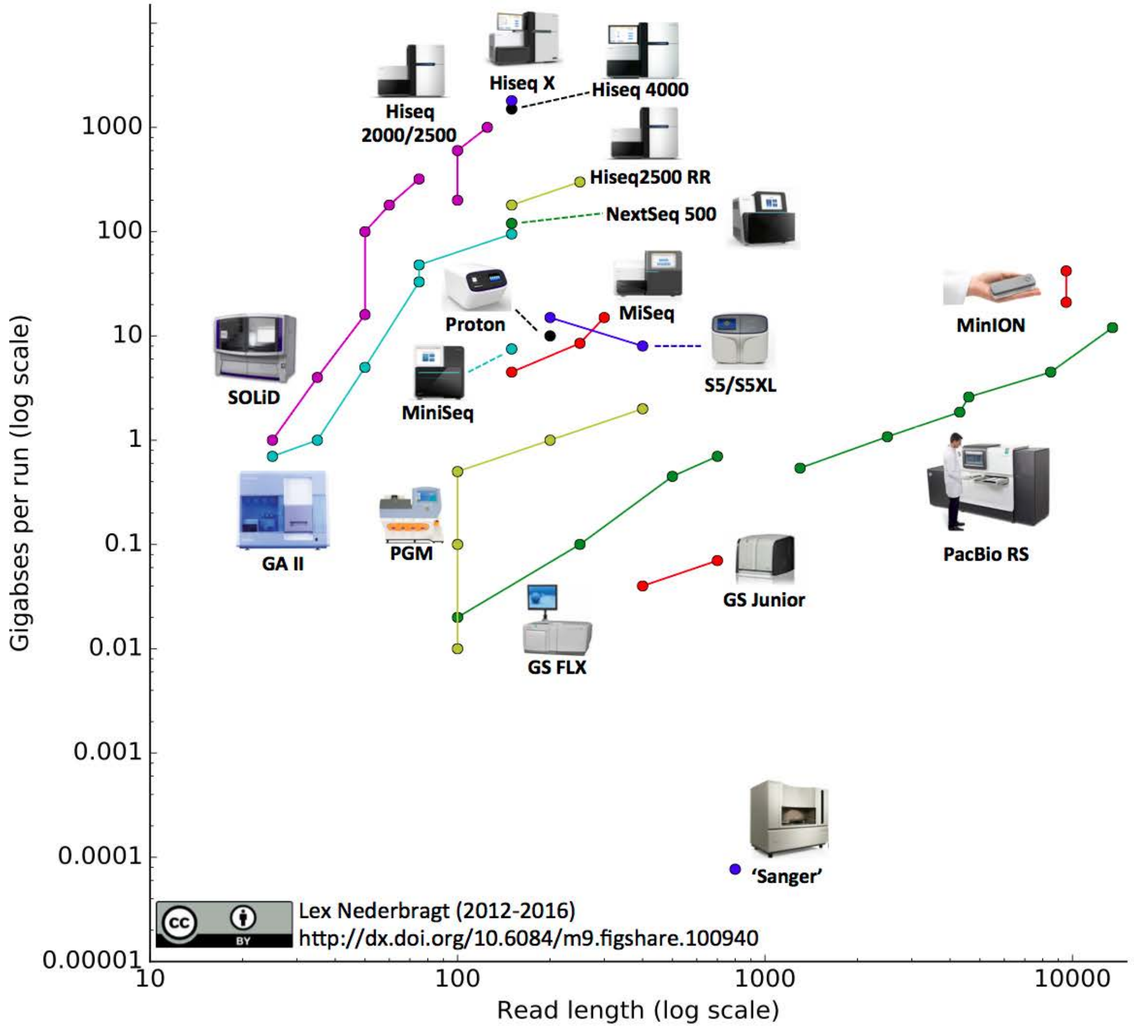
ATGCTACTTG

Genome Sequence



Genomic Epidemiology

Sequencing is likely no longer the bottleneck — it is analysis



Bioinformatics platforms

Commercial “Point and click”

- CLC Genomics
- Geneious Prime

Free “Point and Click”

- UGENE
- MEGA

Web-based pipelines

- Galaxy Server
- Terra (Google cloud)
- Illumina Basespace

Command line

Thousands of individual programs

RAW SEQUENCE DATA FILES

Illumina

fastq

Nanopore

fast5

Ion Torrent

bam

Fastq format

```
1  @SEQ_ID
2  GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
3  +
4  !''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

Line 1 begins with a '@' character and is followed by a sequence identifier and an optional description (like a FASTA title line).

Line 2 is the raw sequence letters.

Line 3 begins with a '+' character and is optionally followed by the same sequence identifier (and any description) again.

Line 4 encodes the quality values for the sequence in Line 2, and must contain the same number of symbols as letters in the sequence.

Fastq format

fastq header format (version > 1.8)

Sequence Header +Sequence ID

a b c d e f g h i j k

@HWI-ST486:166:C06K9ACXX:7:1101:1443:1995 1:N:0:ACAGTG

a. unique instrument name
b. run id
c. flowcell id
d. flowcell lane
e. tile number within the flowcell lane
f. x-coordinate of the cluster within the tile
g. y-coordinate of the cluster within the tile

h. the member of a pair, 1 or 2 (paired-end or mate-pair reads only)
i. Y if the read fails filter (read is bad), N otherwise
j. 0 when no control bits are on
k. index sequence

https://www.gdc-docs.ethz.ch/MDA/site/getdata/

Fastq format

Each sequence read is represented by 4 lines

```
@A00178:71:HGT77DSXX:1:2171:17707:8077 2:N:0:ACAGCAAC+GTTGCTGT
GAAGAAAAGAAGGACACAGAGGAGGGAAAGGTTGAGGAAATTGATGAAGAGAAGGAAGAGAAAGAGAAGAAAAAGAAGACGATCAAGGAGGTTT
+
FFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:1507:30291:23422 1:N:0:ACAGCAAC+GTTGCTGT
ACATAGAGCTTGATGTTGTTGGCCTTCTTCCTGGTGTCGAAGAGGTCAAAGGGGGGCCTCTTGGGGACAAAAAGGACAGCCTTGAACTCAAGCT
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:1507:30291:23422 2:N:0:ACAGCAAC+GTTGCTGT
CTGGATGAGGAAGCCTGAGGAGATCACCAAGGAGGAGTATGCTGCTTTCTATAAAAGCTTGACAAATGACTGGGAAGAGCATCTGGCTGTCAAG
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:2413:22806:35790 1:N:0:ACAGCAAC+GTTGCTGT
GCTTGATGTTGTTGGCCTTCTTCCTGGTGTCGAAGAGGTCAAAGGGGGGCCTCTTGGGGACAAAAAGGACAGCCTTGAACTCAAGCTGCCCCTC
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:2413:22806:35790 2:N:0:ACAGCAAC+GTTGCTGT
GAGAAGAAAAAGAAGACGATCAAGGAGGTTTCTCATGAATGGTCCTTGATCAACAAGCAGAAACCTATCTGGATGAGGAAGCCTGAGGAGATCA
+
F:FF:FFFFFFFF,:FFFFFFFF:FFFFFFFFFFFFF:F:FFFFFFF:FFFFFFFFFFFFFFFF,:FFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:2354:5620:8876 1:N:0:ACAGCAAC+GTTGCTGT
ATGTTGTTGGCCTTCTTCCTGGTGTCGAAGAGGTCAAAGGGGGGCCTCTTGGGGACAAAAAGGACAGCCTTGAACTCAAGCTGCCCCTCTACAG
+
FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:2354:5620:8876 2:N:0:ACAGCAAC+GTTGCTGT
AGAAGGAAGAGAAAGAGAAGAAAAAGAAGACGATCAAGGAGGTTTCTCATGAATGGTCCTTGATCAACAAGCAGAAACCTATCTGGATGAGGAA
+
FFFFFFF,FFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
@A00178:71:HGT77DSXX:1:1560:6741:9815 1:N:0:ACAGCAAC+GTTGCTGT
GCAGGATTTTACCATGATCGACTACTTTTTGTCATGCCCAGAGAAGCTAGATTTTGCCAATGATGTTTATAGACCATTTAACGTTTCGCCAAGC
+
FFFFFF:FFFFFFFFFFFFFFF:FFFFFFFF:FFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF
```

1. Fastq header

2. The read sequence

3. Sequence/quality line separator

```
@ML-P2-14:9:000H003HG:1:11102:17290:1073 1:N:0:TCCTGAGC+GCGATCTA
TTTGGTAACAGCATGAATTATTCTAGCCACTAAAACTCTATGAACATCTTGTGAAGGTTTCAGATAGAGCCTGAAGTACACAGAGAACAATTCTTAAAAAA
+
AAAAAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE<AEEEEEEE
```

4. Sequence quality. There is one character for each nucleotide. The characters relate to a sequence quality score e.g. how likely is the nucleotide correct?
Known as **Phred score**

Quality score interpretation

$$Q = -10 \log_{10} P \quad \Longrightarrow \quad P = 10^{\frac{-Q}{10}}$$

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10000	99.99%
50	1 in 100000	99.999%

The quality (Q), also called Phred score, is the probability (P) that the corresponding basecall is incorrect.

fast5 format

Binary file (not human readable)

Contains:

- Sequence of a read
- Raw signal data from pore
- Additional log files

Typically convert fast5 to fastq for downstream analyses

BAM format for read data

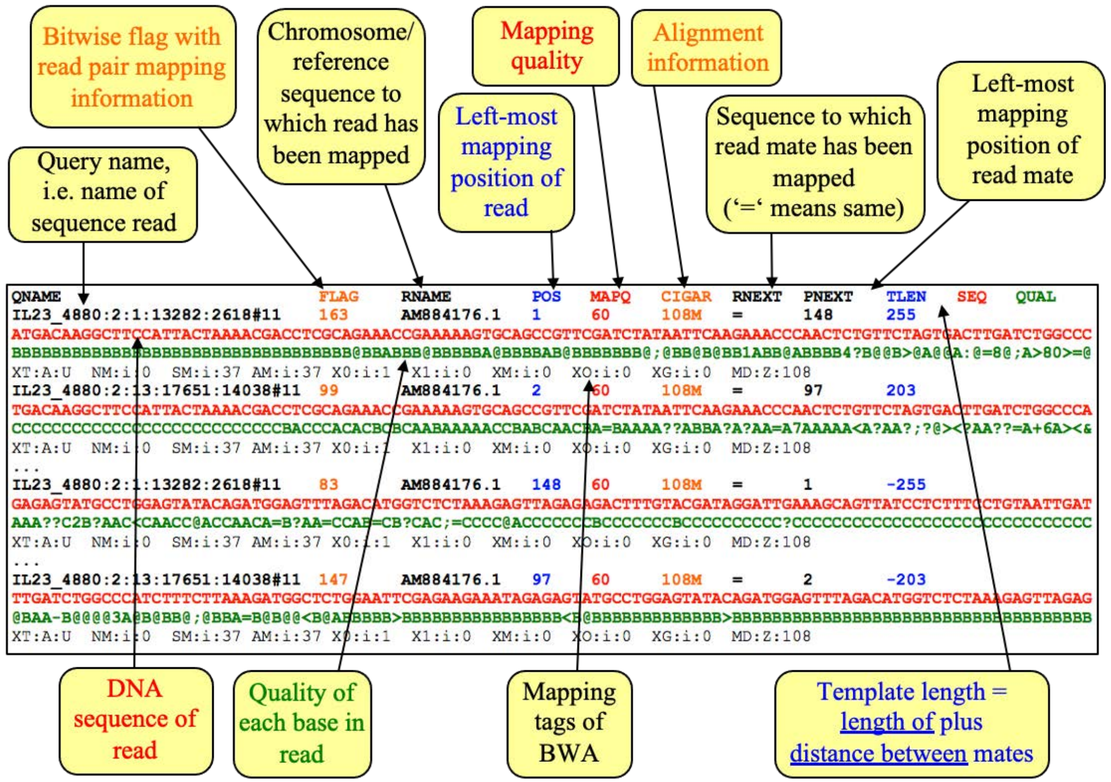
Binary Alignment Map format

Binary conversion of the Sequence Alignment Map (SAM) file

Typically convert bam to fastq for downstream analyses

File format: SAM / BAM (each line: one aligned sequence read)

The SAM/BAM file format is very powerful. It is unlikely that you will need to work with the contents of a SAM/BAM file directly, but it is very informative to visualize it in a viewer and it is a great format to do further analysis with. The format specifications are at http://samtools.sourceforge.net/SAM1.pdf. Below is a brief overview of the information contained in such files.



Fasta format

1. Each entry begins with **'>'**

2. Name of sequence directly after '>'

3. Everything after name is called **Description**

```
>NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome
ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAA
CGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAAC
TAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTG
TTGCAGCCGATCATCAGCACATCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTC
CCTGGTTTCAACGAGAAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTAC
GTGGCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGG
CTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTCATCAAACGTTCGGAT
GCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTC
GTAGTGGTGAGACACTTGGTGTCCTTGTCCCTCATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCT
TCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTA
GGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTAAACATAGCAGTGGTG
TTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGCATACACTCGCTATGTCGATAACAACTTCTGTGG
CCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCACTTTG
TCCGAACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCATGAAATTG
CTTGGTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATTAAATTGGCAAAGAA
ATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCCATAATCAAGACTATTCAA
CCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCAC
CAAATGAATGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTGGTGAAACTTCATGGCA
GACGGGCGATTTTGTTAAAGCCACTTGCGAATTTTGTGGCACTGAGAATTTGACTAAAGAAGGTGCCACT
ACTTGTGGTTACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATTCAGAAGTAG
GACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAAAACCATTCTTCGTAAGGGTGGTCG
CACTATTGCCTTTGGAGGCTGTGTGTTCTCTTATGTTGGTTGCCATAACAAGTGTGCCTATTGGGTTCCA
```

4. Sequence (nucleotides or AA)

Can be one line or have line break every 60-80 characters (like here)

File name extensions:

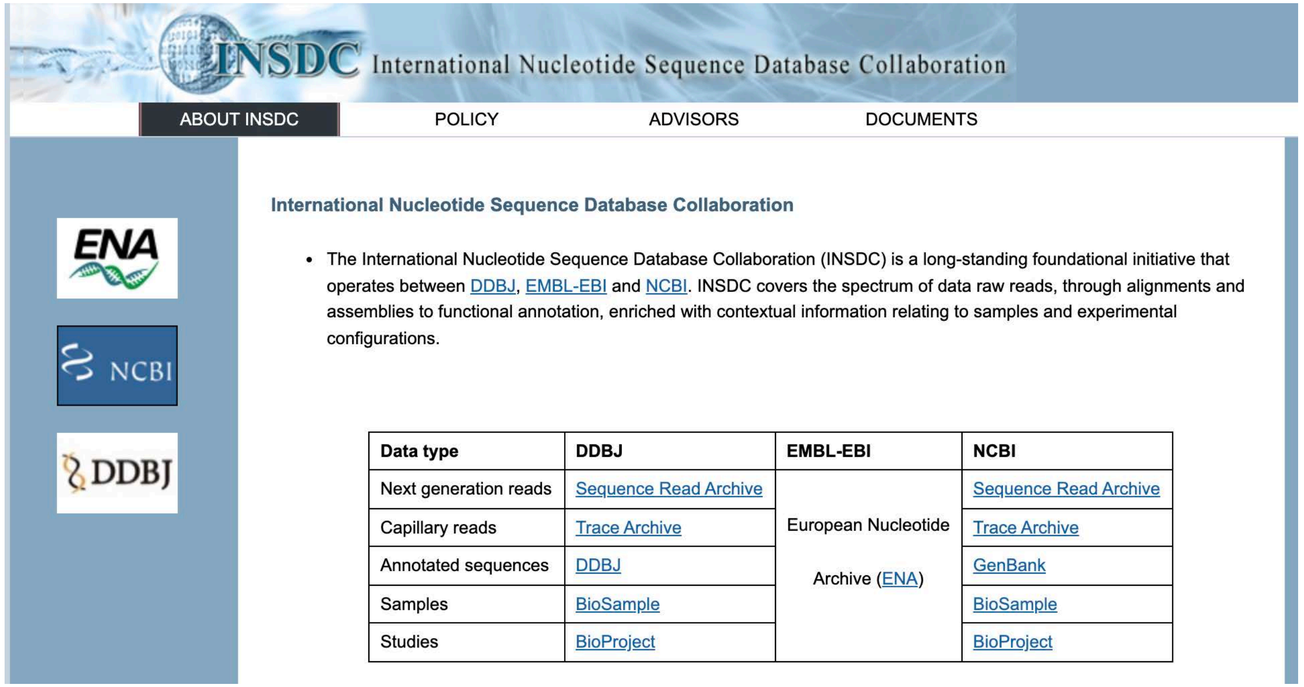
- .fnt (nucleotide)
- .fna (nucleotide)
- .faa (amino acid)
- .fasta
- .fa
- .fas

Two common paths to generating genome fasta files

GENOME ANNOTATION FILES

Genome annotation files

- European Nucleotide Archive (ENA) : EMBL
- NIH / NCBI : GenBank
- General Feature File (GFF)



INSDC International Nucleotide Sequence Database Collaboration

ABOUT INSDC | POLICY | ADVISORS | DOCUMENTS

ENA

NCBI

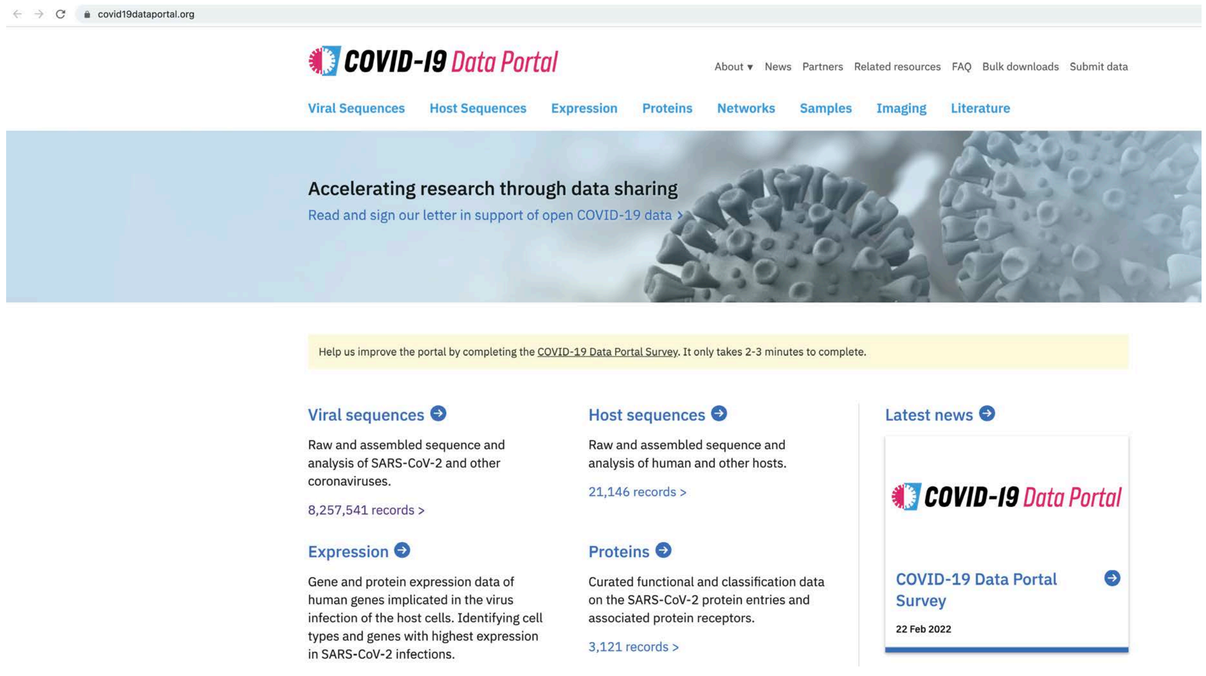
DDBJ

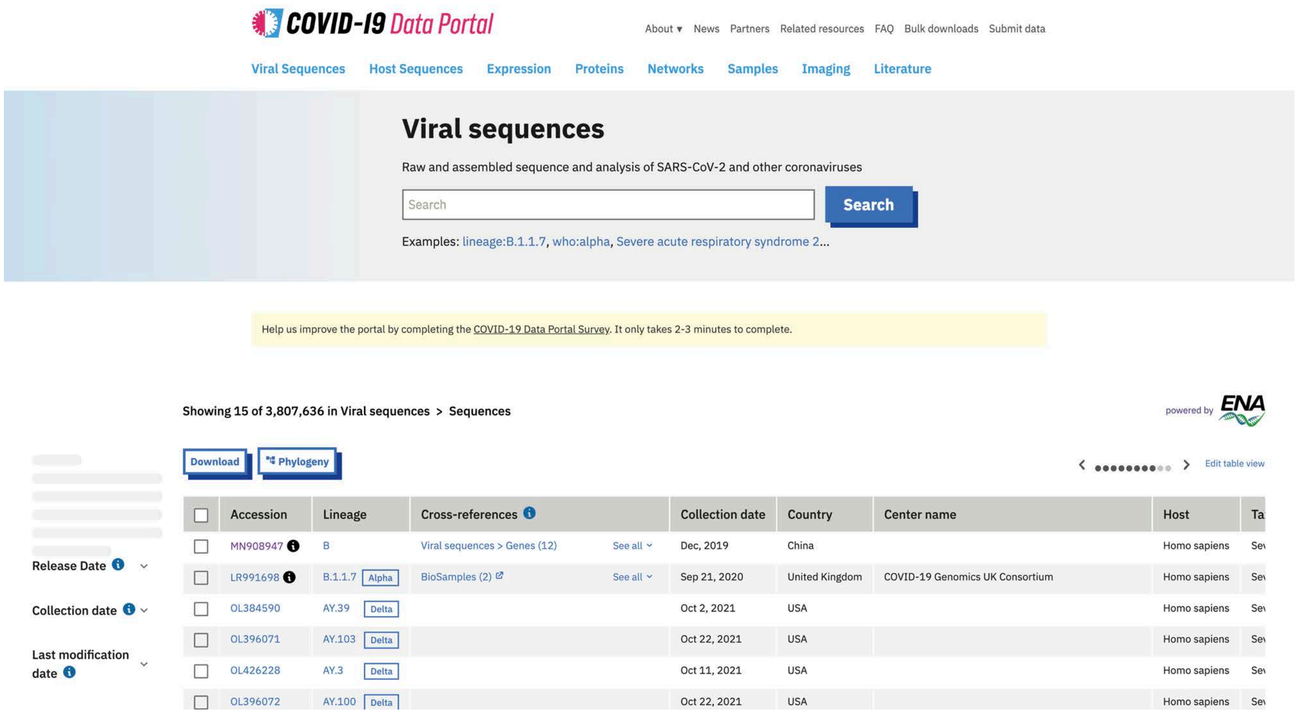
International Nucleotide Sequence Database Collaboration

- The International Nucleotide Sequence Database Collaboration (INSDC) is a long-standing foundational initiative that operates between DDBJ, EMBL-EBI and NCBI. INSDC covers the spectrum of data raw reads, through alignments and assemblies to functional annotation, enriched with contextual information relating to samples and experimental configurations.

Data type	DDBJ	EMBL-EBI	NCBI
Next generation reads	Sequence Read Archive	European Nucleotide Archive (ENA)	Sequence Read Archive
Capillary reads	Trace Archive		Trace Archive
Annotated sequences	DDBJ		GenBank
Samples	BioSample		BioSample
Studies	BioProject		BioProject

COVID-19 Data Portal — ENA





EMBL format from EBI

Two-character line code indicates the type of information contained in the line

Feature Key

Header

Annotation

Sequence

```
ID   MN908947; SV 3; linear; genomic RNA; STD; VRL; 29903 BP.
XX
AC   MN908947;
XX
DT   13-JAN-2020 (Rel. 143, Created)
DT   19-MAR-2020 (Rel. 144, Last updated, Version 6)
XX
DE   Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1,
DE   complete genome.
XX
KW   .
XX
OS   Severe acute respiratory syndrome coronavirus 2
OC   Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
OC   Orthocoronavirinae; Betacoronavirus; Sarbecovirus.
XX
RN   [1]
RP   1-29903
RX   PUBMED; 32015508.
RA   Wu F., Zhao S., Yu B., Chen Y.M., Wang W., Song Z.G., Hu Y., Tao Z.W.,
RA   Tian J.H., Pei Y.Y., Yuan M.L., Zhang Y.L., Dai F.H., Liu Y., Wang Q.M.,
RA   Zheng J.J., Xu L., Holmes E.C., Zhang Y.Z.;
RT   "A new coronavirus associated with human respiratory disease in China";
RL   Nature 579(7798):265-269(2020).
XX
RN   [2]
RP   1-29903
RA   Wu F., Zhao S., Yu B., Chen Y.-M., Wang W., Hu Y., Song Z.-G., Tao Z.-W.,
RA   Tian J.-H., Pei Y.-Y., Yuan M.L., Zhang Y.-L., Dai F.-H., Liu Y.,
RA   Wang Q.-M., Zheng J.-J., Xu L., Holmes E.C., Zhang Y.-Z.;
RT   ;
RL   Submitted (05-JAN-2020) to the INSDC.
RL   Shanghai Public Health Clinical Center & School of Public Health, Fudan
RL   University, Shanghai, China
XX
DR   MD5; 105c82802b67521950854a851fc6eefd.
XX
CC   On Jan 17, 2020 this sequence version replaced MN908947.2.
CC   ##Assembly-Data-START##
CC   Assembly Method       :: Megahit v. V1.1.3
CC   Sequencing Technology :: Illumina
CC   ##Assembly-Data-END##
XX
FH   Key             Location/Qualifiers
FH
FT   source          1..29903
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FT                   /host="Homo sapiens"
FT                   /isolate="Wuhan-Hu-1"
FT                   /mol_type="genomic RNA"
FT                   /country="China"
FT                   /collection_date="Dec-2019"
FT                   /db_xref="taxon:2697049"
FT   5'UTR           1..265
FT   gene            266..21555
FT                   /gene="orf1ab"
FT   CDS             join(266..13468,13468..21555)
FT                   /codon_start=1
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FT                   /gene="orf1ab"
FT                   /product="orf1ab polyprotein"
FT                   /note="pp1ab; translated by -1 ribosomal frameshift"
FT                   /protein_id="QHD43415.1"
FT                   /translation="MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQH
FT                   LKDGTCGLVEVEKGVLPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQYGRSGETL
FT                   GVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFDLGDELGTDPYEDFQENWNT
FT                   KHSSGVTRELMRELNGGAYTRYVDNNFCGPDGYPLECIKDLLARAGKASCTLSEQLDFI
XX
SQ   Sequence 29903 BP; 8954 A; 5492 C; 5863 G; 9594 T; 0 other;
     attaaaggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct        60
     gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcatgc ttagtgcact       120
     cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgtctatc       180
     ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctaggttt       240
     cgtccgggtg tgaccgaaag gtaagatgga gagccttgtc cctggtttca acgagaaaac       300
     acacgtccaa ctcagtttgc ctgttttaca ggttcgcgac gtgctcgtac gtggctttgg       360
     agactccgtg gaggaggtct tatcagaggc acgtcaacat cttaaagatg gcacttgtgg       420
```

NIH / NCBI Virus

National Library of Medicine
National Center for Biotechnology Information

Log in

COVID-19 Information
Public health information (CDC) | Research information (NIH) | SARS-CoV-2 data (NCBI) | Prevention and treatment information (HHS) | Español

NCBI Virus
Sequences for discovery

About Us | Find Data | Help | How to Participate | Submit Sequences | Contact Us

SARS-CoV-2 Data Hub

Download

Quick Links: Betacoronavirus BLAST | CDC Outbreak Information | SARS-CoV-2 Articles in PubMed | SRA Data | NCBI SARS-CoV-2 Resources | Datasets command line

Tabular View | Dashboard Visualizations | Mutations in SRA | Complete Tree

Selected Results: 0 | Align | Build Phylogenetic Tree

Refine Results — Reset

- Virus
  - Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), taxid:2697049
- Accession
- Sequence Length
- Ambiguous Characters
- Sequence Type
- RefSeq Genome Completeness
- Nucleotide Completeness
- Pango lineage
- Random Sampling New!
- Isolate
- Proteins
- Provirus
- Geographic Region
- Host
- Submitters
- Isolation Source

Expand Table

Nucleotide (4,083,187) | Protein (24,012,480) | RefSeq Genome (1)

Select Columns

Accession	Submitters	Release Date	Pangolin	Isolate	Species	Molecule type	Length	Geo Location	USA	Host	Isolation Source	Collection Date
NC_045512 RefSeq	Wu,F., et al.	2020-01-13	B	Wuhan-Hu-1	Severe acute respiratory syndrome-r…	ssRNA(+)	29903	China		Homo sapiens		2019-12
OM840138	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0847	Severe acute respiratory syndrome-r…	ssRNA(+)	29781	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840139	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0850	Severe acute respiratory syndrome-r…	ssRNA(+)	29808	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840140	Andrews,K.R., et al.	2022-02-27	B.1	ID-UI-IIDS-U0852	Severe acute respiratory syndrome-r…	ssRNA(+)	29780	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840141	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0853	Severe acute respiratory syndrome-r…	ssRNA(+)	29717	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840142	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0856	Severe acute respiratory syndrome-r…	ssRNA(+)	29775	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840143	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0857	Severe acute respiratory syndrome-r…	ssRNA(+)	29717	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840144	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0862	Severe acute respiratory syndrome-r…	ssRNA(+)	29779	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840145	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0863	Severe acute respiratory syndrome-r…	ssRNA(+)	29808	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840146	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0864	Severe acute respiratory syndrome-r…	ssRNA(+)	29808	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840147	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0866	Severe acute respiratory syndrome-r…	ssRNA(+)	29780	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840148	Andrews,K.R., et al.	2022-02-27	B.1	ID-UI-IIDS-U0867	Severe acute respiratory syndrome-r…	ssRNA(+)	29780	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840149	Andrews,K.R., et al.	2022-02-27	B.1	ID-UI-IIDS-U0870	Severe acute respiratory syndrome-r…	ssRNA(+)	29779	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840150	Andrews,K.R., et al.	2022-02-27	B.1.2	ID-UI-IIDS-U0872	Severe acute respiratory syndrome-r…	ssRNA(+)	29779	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11
OM840151	Andrews,K.R., et al.	2022-02-27	B.1	ID-UI-IIDS-U0877	Severe acute respiratory syndrome-r…	ssRNA(+)	29808	USA: Moscow, Idaho	ID	Homo sapiens	oronasopharynx	2020-11-11

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GenBank format from NCBI

```
LOCUS       NC_045512              29903 bp ss-RNA     linear   VRL 18-JUL-2020
DEFINITION  Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1,
            complete genome.
ACCESSION   NC_045512
VERSION     NC_045512.2
DBLINK      BioProject: PRJNA485481
KEYWORDS    RefSeq.
SOURCE      Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
  ORGANISM  Severe acute respiratory syndrome coronavirus 2
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;
            Nidovirales; Cornidovirineae; Coronaviridae; Orthocoronavirinae;
            Betacoronavirus; Sarbecovirus.
REFERENCE   1  (bases 1 to 29903)
  AUTHORS   Wu,F., Zhao,S., Yu,B., Chen,Y.M., Wang,W., Song,Z.G., Hu,Y.,
            Tao,Z.W., Tian,J.H., Pei,Y.Y., Yuan,M.L., Zhang,Y.L., Dai,F.H.,
            Liu,Y., Wang,Q.M., Zheng,J.J., Xu,L., Holmes,E.C. and Zhang,Y.Z.
  TITLE     A new coronavirus associated with human respiratory disease in
            China
  JOURNAL   Nature 579 (7798), 265-269 (2020)
   PUBMED   32015508
  REMARK    Erratum:[Nature. 2020 Apr;580(7803):E7. PMID: 32296181]
REFERENCE   2  (bases 13476 to 13503)
  AUTHORS   Baranov,P.V., Henderson,C.M., Anderson,C.B., Gesteland,R.F.,
            Atkins,J.F. and Howard,M.T.
  TITLE     Programmed ribosomal frameshifting in decoding the SARS-CoV genome
  JOURNAL   Virology 332 (2), 498-510 (2005)
   PUBMED   15680415
REFERENCE   3  (bases 29728 to 29768)
  AUTHORS   Robertson,M.P., Igel,H., Baertsch,R., Haussler,D., Ares,M. Jr. and
            Scott,W.G.
  TITLE     The structure of a rigorously conserved RNA element within the SARS
            virus genome
  JOURNAL   PLoS Biol. 3 (1), e5 (2005)
   PUBMED   15630477
REFERENCE   4  (bases 29609 to 29657)
  AUTHORS   Williams,G.D., Chang,R.Y. and Brian,D.A.
  TITLE     A phylogenetically conserved hairpin-type 3' untranslated region
            pseudoknot functions in coronavirus RNA replication
  JOURNAL   J. Virol. 73 (10), 8349-8355 (1999)
   PUBMED   10482585
REFERENCE   5  (bases 1 to 29903)
  CONSRTM   NCBI Genome Project
  TITLE     Direct Submission
  JOURNAL   Submitted (17-JAN-2020) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
REFERENCE   6  (bases 1 to 29903)
  AUTHORS   Wu,F., Zhao,S., Yu,B., Chen,Y.-M., Wang,W., Hu,Y., Song,Z.-G.,
            Tao,Z.-W., Tian,J.-H., Pei,Y.-Y., Yuan,M.L., Zhang,Y.-L.,
            Dai,F.-H., Liu,Y., Wang,Q.-M., Zheng,J.-J., Xu,L., Holmes,E.C. and
            Zhang,Y.-Z.
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JAN-2020) Shanghai Public Health Clinical Center &
            School of Public Health, Fudan University, Shanghai, China
COMMENT     REVIEWED REFSEQ: This record has been curated by NCBI staff. The
            reference sequence is identical to MN908947.
            On Jan 17, 2020 this sequence version replaced NC_045512.1.
            Annotation was added using homology to SARS-CoV NC_004718.3. ###
            Formerly called 'Wuhan seafood market pneumonia virus.' If you have
            questions or suggestions, please email us at info@ncbi.nlm.nih.gov
            and include the accession number NC_045512.### Protein structures
            can be found at
            https://www.ncbi.nlm.nih.gov/structure/?term=sars-cov-2.### Find
            all other Severe acute respiratory syndrome coronavirus 2
            (SARS-CoV-2) sequences at
            https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/

            ##Assembly-Data-START##
            Assembly Method       :: Megahit v. V1.1.3
            Sequencing Technology :: Illumina
            ##Assembly-Data-END##
            COMPLETENESS: full length.
FEATURES             Location/Qualifiers
     source          1..29903
                     /organism="Severe acute respiratory syndrome coronavirus
                     2"
                     /mol_type="genomic RNA"
                     /isolate="Wuhan-Hu-1"
                     /host="Homo sapiens"
                     /db_xref="taxon:2697049"
                     /country="China"
                     /collection_date="Dec-2019"
     5'UTR           1..265
     gene            266..21555
                     /gene="ORF1ab"
                     /locus_tag="GU280_gp01"
                     /db_xref="GeneID:43740578"
     CDS             join(266..13468,13468..21555)
                     /gene="ORF1ab"
                     /locus_tag="GU280_gp01"
                     /ribosomal_slippage
                     /note="pp1ab; translated by -1 ribosomal frameshift"
                     /codon_start=1
                     /product="ORF1ab polyprotein"
                     /protein_id="YP_009724389.1"
                     /db_xref="GeneID:43740578"
                     /translation="MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQ
                     HLKDGTCGLVEVEKGVLPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQYGRSGE
                     TLGVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFDLGDELGTDPYEDFQEN
                     WNTKHSSGVTRELMRELNGGAYTRYVDNNFCGPDGYPLECIKDLLARAGKASCTLSEQ
```

Header

Annotation

```
ORIGIN
        1 attaaaggtt tataccttcc caggtaacaa accaaccaac tttcgatctc ttgtagatct
       61 gttctctaaa cgaactttaa aatctgtgtg gctgtcactc ggctgcatgc ttagtgcact
      121 cacgcagtat aattaataac taattactgt cgttgacagg acacgagtaa ctcgtctatc
      181 ttctgcaggc tgcttacggt ttcgtccgtg ttgcagccga tcatcagcac atctaggttt
      241 cgtccgggtg tgaccgaaag gtaagatgga gagccttgtc cctggtttca acgagaaaac
```

Sequence

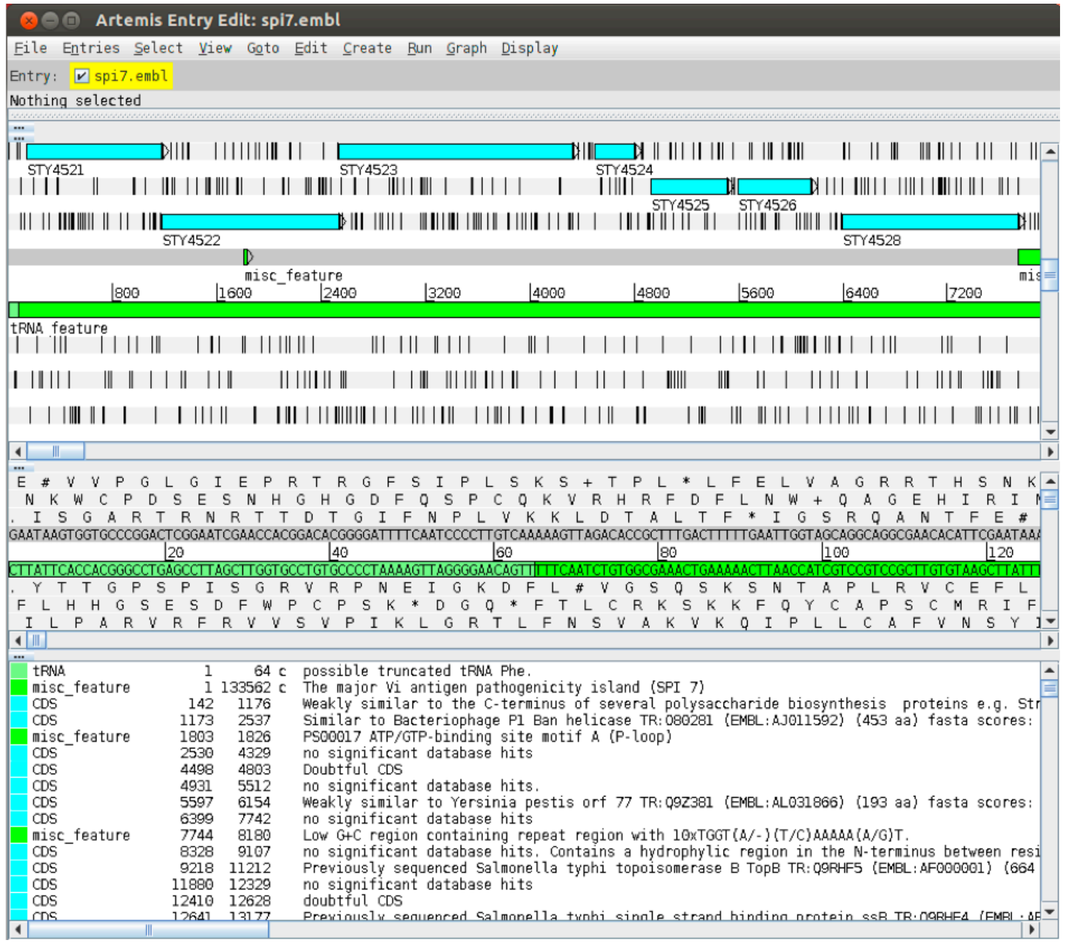
General Feature File (GFF)

```
##sequence-region NC_045512.2 1 29903
##species https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2697049
NC_045512.2	RefSeq	region	1	29903	.	+	.	ID=NC_045512.2:1..29903;Dbxref=taxon:2697049;collection-date=Dec-2019;country=China;gb-acronym=SARS-CoV-2;gbkey=Src;genome=genomic;isolate=Wuhan-Hu-1;mol_type=genomic RNA;nat-host=Homo sapiens;old-name=Wuhan seafood market pneumonia virus
NC_045512.2	RefSeq	five_prime_UTR	1	265	.	+	.	ID=id-NC_045512.2:1..265;gbkey=5'UTR
NC_045512.2	RefSeq	gene	266	21555	.	+	.	ID=gene-GU280_gp01;Dbxref=GeneID:43740578;Name=ORF1ab;gbkey=Gene;gene=ORF1ab;gene_biotype=protein_coding;locus_tag=GU280_gp01
NC_045512.2	RefSeq	CDS	266	13468	.	+	0	ID=cds-YP_009724389.1;Parent=gene-GU280_gp01;Dbxref=Genbank:YP_009724389.1,GeneID:43740578;Name=YP_009724389.1;Note=pp1ab%3B translated by -1 ribosomal frameshift;exception=ribosomal slippage;gbkey=CDS;gene=ORF1ab;locus_tag=GU280_gp01;product=ORF1ab polyprotein;protein_id=YP_009724389.1
NC_045512.2	RefSeq	CDS	13468	21555	.	+	0	ID=cds-YP_009724389.1;Parent=gene-GU280_gp01;Dbxref=Genbank:YP_009724389.1,GeneID:43740578;Name=YP_009724389.1;Note=pp1ab%3B translated by -1 ribosomal frameshift;exception=ribosomal slippage;gbkey=CDS;gene=ORF1ab;locus_tag=GU280_gp01;product=ORF1ab polyprotein;protein_id=YP_009724389.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	266	805	.	+	.	ID=id-YP_009724389.1:1..180;Note=nsp1%3B produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=leader protein;protein_id=YP_009725297.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	806	2719	.	+	.	ID=id-YP_009724389.1:181..818;Note=produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp2;protein_id=YP_009725298.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	2720	8554	.	+	.	ID=id-YP_009724389.1:819..2763;Note=former nsp1%3B conserved domains are: N-terminal acidic (Ac)%2C predicted phosphoesterase%2C papain-like proteinase%2C Y-domain%2C transmembrane domain 1 (TM1)%2C adenosine diphosphate-ribose 1''-phosphatase (ADRP)%3B produced by both
NC_045512.2	RefSeq	mature_protein_region_of_CDS	8555	10054	.	+	.	ID=id-YP_009724389.1:2764..3263;Note=nsp4B_TM%3B contains transmembrane domain 2 (TM2)%3B produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp4;protein_id=YP_009725300.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	10055	10972	.	+	.	ID=id-YP_009724389.1:3264..3569;Note=nsp5A_3CLpro and nsp5B_3CLpro%3B main proteinase (Mpro)%3B mediates cleavages downstream of nsp4. 3D structure of the SARSr-CoV homolog has been determined (Yang et al.%2C 2003)%3B produced by both pp1a and pp1ab;Parent=cds-YP_009724
NC_045512.2	RefSeq	mature_protein_region_of_CDS	10973	11842	.	+	.	ID=id-YP_009724389.1:3570..3859;Note=nsp6_TM%3B putative transmembrane domain%3B produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp6;protein_id=YP_009725302.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	11843	12091	.	+	.	ID=id-YP_009724389.1:3860..3942;Note=produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp7;protein_id=YP_009725303.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	12092	12685	.	+	.	ID=id-YP_009724389.1:3943..4140;Note=produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp8;protein_id=YP_009725304.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	12686	13024	.	+	.	ID=id-YP_009724389.1:4141..4253;Note=ssRNA-binding protein%3B produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp9;protein_id=YP_009725305.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	13025	13441	.	+	.	ID=id-YP_009724389.1:4254..4392;Note=nsp10_CysHis%3B formerly known as growth-factor-like protein (GFL)%3B produced by both pp1a and pp1ab;Parent=cds-YP_009724389.1;gbkey=Prot;product=nsp10;protein_id=YP_009725306.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	13442	13468	.	+	.	ID=id-YP_009724389.1:4393..5324;Note=nsp12%3B NiRAN and RdRp%3B produced by pp1ab only;Parent=cds-YP_009724389.1;gbkey=Prot;product=RNA-dependent RNA polymerase;protein_id=YP_009725307.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	13468	16236	.	+	.	ID=id-YP_009724389.1:4393..5324;Note=nsp12%3B NiRAN and RdRp%3B produced by pp1ab only;Parent=cds-YP_009724389.1;gbkey=Prot;product=RNA-dependent RNA polymerase;protein_id=YP_009725307.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	16237	18039	.	+	.	ID=id-YP_009724389.1:5325..5925;Note=nsp13_ZBD%2C nsp13_TB%2C and nsp_HEL1core%3B zinc-binding domain (ZD)%2C NTPase/helicase domain (HEL)%2C RNA 5'-triphosphatase%3B produced by pp1ab only;Parent=cds-YP_009724389.1;gbkey=Prot;product=helicase;protein_id=YP_009725308.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	18040	19620	.	+	.	ID=id-YP_009724389.1:5926..6452;Note=nsp14A2_ExoN and nsp14B_NMT%3B produced by pp1ab only;Parent=cds-YP_009724389.1;gbkey=Prot;product=3'-to-5' exonuclease;protein_id=YP_009725309.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	19621	20658	.	+	.	ID=id-YP_009724389.1:6453..6798;Note=nsp15-A1 and nsp15B-NendoU%3B produced by pp1ab only;Parent=cds-YP_009724389.1;gbkey=Prot;product=endoRNAse;protein_id=YP_009725310.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	20659	21552	.	+	.	ID=id-YP_009724389.1:6799..7096;Note=nsp16_OMT%3B 2'-o-MT%3B produced by pp1ab only;Parent=cds-YP_009724389.1;gbkey=Prot;product=2'-O-ribose methyltransferase;protein_id=YP_009725311.1
NC_045512.2	RefSeq	CDS	266	13483	.	+	0	ID=cds-YP_009725295.1;Parent=gene-GU280_gp01;Dbxref=Genbank:YP_009725295.1,GeneID:43740578;Name=YP_009725295.1;Note=pp1a;gbkey=CDS;gene=ORF1ab;locus_tag=GU280_gp01;product=ORF1a polyprotein;protein_id=YP_009725295.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	266	805	.	+	.	ID=id-YP_009725295.1:1..180;Note=nsp1%3B produced by both pp1a and pp1ab;Parent=cds-YP_009725295.1;gbkey=Prot;product=leader protein;protein_id=YP_009742608.1
NC_045512.2	RefSeq	mature_protein_region_of_CDS	806	2719	.	+	.	ID=id-YP_009725295.1:181..818;Note=produced by both pp1a and pp1ab;Parent=cds-YP_009725295.1;gbkey=Prot;product=nsp2;protein_id=YP_009742609.1
```

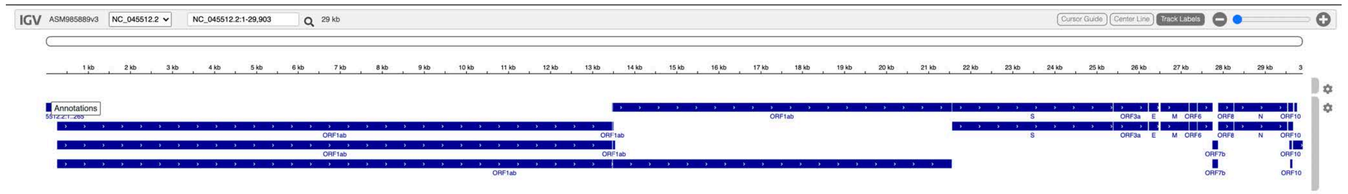
General GFF3 structure

Position index	Position name	Description
1	seqid	The name of the sequence where the feature is located.
2	source	Keyword identifying the source of the feature, like a program (e.g. Augustus or RepeatMasker) or an organization (like TAIR).
3	type	The feature type name, like "gene" or "exon". In a well structured GFF file, all the children features always follow their parents in a single block (so all exons of a transcript are put after their parent "transcript" feature line and before any other parent transcript line). In GFF3, all features and their relationships should be compatible with the standards released by the Sequence Ontology Project.
4	start	Genomic start of the feature, with a **1-base offset**. This is in contrast with other 0-offset half-open sequence formats, like BED.
5	end	Genomic end of the feature, with a **1-base offset**. This is the same end coordinate as it is in 0-offset half-open sequence formats, like BED.[*citation needed*]
6	score	Numeric value that generally indicates the confidence of the source in the annotated feature. A value of "." (a dot) is used to define a null value.
7	strand	Single character that indicates the strand of the feature; it can assume the values of "+" (positive, or 5'->3'), "-", (negative, or 3'->5'), "." (undetermined).
8	phase	phase of CDS features; it can be either one of 0, 1, 2 (for CDS features) or "." (for everything else). See the section below for a detailed explanation.
9	attributes	All the other information pertaining to this feature. The format, structure and content of this field is the one which varies the most between the three competing file formats.

Annotation files can be visualized and explored



Artemis



Integrated Genome Viewer
(IGV)

Questions?

Today's Agenda

- Intro talk on NGS data formats
- Bioinformatics Module 1 (~30-40 min)
- Session wrap up & VM install guide

Bioinformatics Module 1

https://domman-genomics.github.io/Oman_NGS/manuals/01_Intro_to_NGS/module_Intro.html

Explore NextClade

-utilize SARS-CoV-2 genomes in **.fasta** format
- Start building an understanding of looking at genome data

Call lineages with Pangolin

- use web based Pangolin to designate Pango lineages