# Linux for Biologists

A Cookbook

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# Contents

This is an exercise in using the command-line to accomplish a task. You will be making use of the commands discussed earlier.

### Task

Given a protein sequence, identify matching sequences from a protein sequence database.

### Approach

Using programs in the NCBI BLAST+ package, you can search a database of sequences using sequence (query) to identify matching sequences.

# 1 Summary of steps

1. Install NCBI BLAST+
2. Download protein query sequence
3. Download protein sequence database and format it
4. Search database using the query sequence

# 2 Get sample data

To proceed, you will need to download the protein query sequence and database used in this exercise.

## 2.1 Download query sequence

The protein query sequence used in this exercise is *Spike glycoprotein* from Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is available from UniProtKB[1] — the protein knowledge base.

The database identifier for this protein is P0DTC2[2]. You can download the sequence in FASTA format from the entry page or using this direct link:

https://www.uniprot.org/uniprot/P0DTC2.fasta

[1] https://uniprot.org/uniprot/

[2] https://www.uniprot.org/uniprot/P0DTC2

## 2.2 Download protein database

The database used in this exercise is UniProtKB Swiss-Prot[3]. It is a manually annotated database of protein sequences with added functional information.

You can download the entire database as a compressed FASTA format file from the downloads[4] page on the website.

[3] https://uniprot.org/uniprot/?query=reviewed:yes

[4] https://www.uniprot.org/downloads

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# Install NCBI BLAST+ package

**Attention:** *This procedure installs software in system paths and so requires administrator privileges.*

NCBI BLAST+ is available in the Linux package repositories. You can install it using `apt`:

```
sudo apt install ncbi-blast+
```

Type `y` when prompted to continue.

```
[sudo] password for user:
Reading package lists... Done
Building dependency tree
Reading state information... Done
The following additional packages will be installed:
  libmbedcrypto3 libmbedtls12 libmbedx509-0 ncbi-data
```

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```
The following NEW packages will be installed:
  libmbedcrypto3 libmbedtls12 libmbedx509-0 ncbi-blast+ ncbi-data
0 upgraded, 5 newly installed, 0 to remove and 0 not upgraded.
Need to get 14.9 MB of archives.
After this operation, 75.0 MB of additional disk space will be used.
Do you want to continue? [Y/n] y
```

# 4

# Download query sequence

You can follow these steps to download the query sequence:

1. Create new directory
2. Change into it
3. Download query sequence
4. View the downloaded sequence (optional)

## 4.1 Create new directory — `mkdir`

To keep the input and output files related to this project together, create a new directory in your home directory using the mkdir command.

```
mkdir -p ~/projects/sars-cov-2
```

Here:

~ is shortcut for home directory.

`-p` creates parent directories if necessary. In this case, the `projects` directory does not exist, so it is also created.

## 4.2 Change directory — cd

Change into the newly created directory using the cd command:

```
cd ~/projects/sars-cov-2
```

## 4.3 Download sequence — `wget`

To download the sequence file, you can use the `wget` command with the link to download as the argument. In this case, the link to download is the URL corresponding to the FASTA format file (see *sample data*):

```
wget https://www.uniprot.org/uniprot/P0DTC2.fasta
```

When the download is complete, you can use the ls command to verify if the file exists:

```
ls -l
```

Output:

```
total 4
-rw-rw-r-- 1 user user 1414 Feb 10 00:00 P0DTC2.fasta
```

## 4.4 View downloaded sequence — cat or less

Since P0DTC2.fasta is in FASTA format — a plain-text format, you can use the cat command to view the file's contents:

```
cat P0DTC2.fasta
```

Output:

```
>sp|P0DTC2|SPIKE_SARS2 Spike glycoprotein OS=Severe acute▯
 ↪respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1▯
 ↪SV=1
MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIAD
YNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPC
NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVN
FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP
GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY
ECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI
SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE
VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC
LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM
QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN
TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA
```

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```
SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA
ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP
LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD
SEPVLKGVKLHYT
```

For more control, you can use the less command instead of `cat`.

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# Download protein sequence database

You can follow these steps to download and prepare the protein sequence database:

1. Create new directory
2. Change into it
3. Download the database archive
4. Uncompress (or extract) the database archive
5. Format the database

## 5.1 Create new directory — `mkdir`

In order to keep all BLAST databases in one location, create a directory to store them using the mkdir command:

```
mkdir ~/databases
```

## 5.2 Change directory — cd

Change into the newly created directory using the cd command:

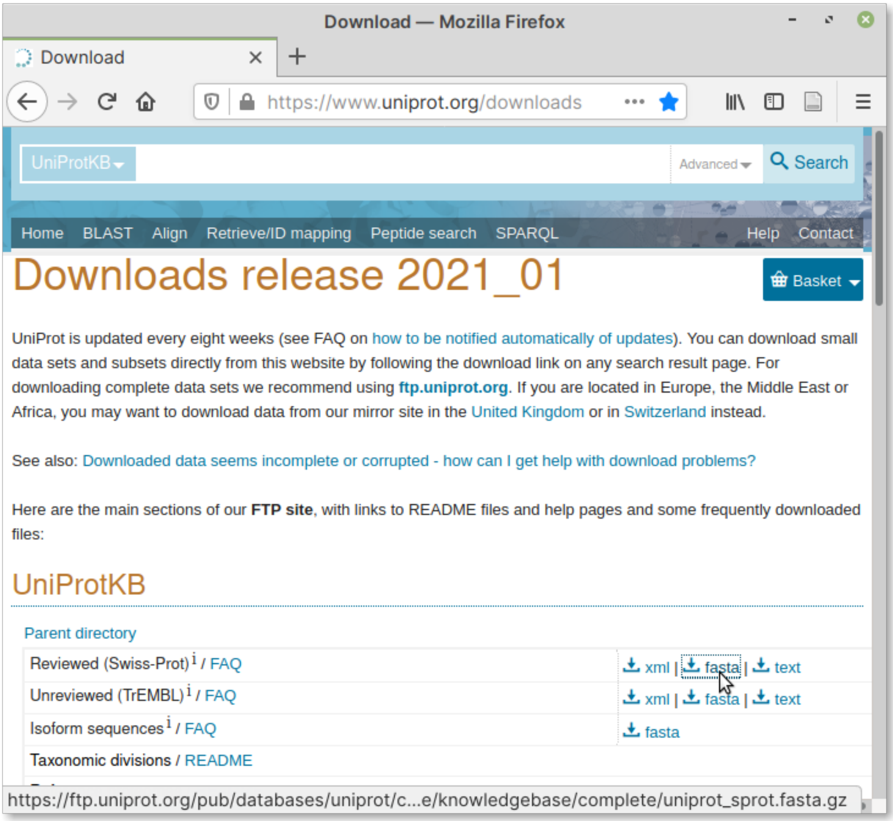
```
cd ~/databases
```

## 5.3 Download the database archive — `wget`

Visit the database downloads[5] page on the UniProt website.

Navigate to the UniProtKB section.

Right-click on the fasta download link corresponding to Reviewed (Swiss-Prot) and then copy it to clipboard (Fig. 1).



Downloads release 2021_01

UniProt is updated every eight weeks (see FAQ on how to be notified automatically of updates). You can download small data sets and subsets directly from this website by following the download link on any search result page. For downloading complete data sets we recommend using **ftp.uniprot.org**. If you are located in Europe, the Middle East or Africa, you may want to download data from our mirror site in the United Kingdom or in Switzerland instead.

See also: Downloaded data seems incomplete or corrupted - how can I get help with download problems?

Here are the main sections of our **FTP site**, with links to README files and help pages and some frequently downloaded files:

UniProtKB

Parent directory

| | |
|---|---|
| Reviewed (Swiss-Prot) i / FAQ | xml \| fasta \| text |
| Unreviewed (TrEMBL) i / FAQ | xml \| fasta \| text |
| Isoform sequences i / FAQ | fasta |
| Taxonomic divisions / README | |

https://ftp.uniprot.org/pub/databases/uniprot/c...e/knowledgebase/complete/uniprot_sprot.fasta.gz

Fig. 1: Download link for Swiss-Prot database

To download the database, you can use the `wget` command with

[5] https://www.uniprot.org/downloads

the link to download as the argument:

```
wget https://ftp.uniprot.org/pub/databases/uniprot/
↪current_release/knowledgebase/complete/uniprot_sprot.
↪fasta.gz
```

When the download is complete, you will find a file named `uniprot_sprot.fasta.gz` in the current directory. You can use the ls command to verify if it exists:

```
ls -lh
```

Output:

```
total 86M
-rw-rw-r-- 1 user user 86M Feb 10 15:00 uniprot_sprot.
↪fasta.gz
```

Since this file is in a compressed format (`.gz`), you will need to uncompress it before proceeding.

## 5.4 Uncompress the database archive — gunzip

To uncompress (or extract) the database archive file downloaded in the previous step, you can use the gunzip command.

**Note:** By default, gunzip will remove the original compressed file after extraction.

If you would like to keep the original file (.gz), you can include the -k (keep input files) option with gunzip.

Provide the file name of the downloaded file as the argument:

```
gunzip uniprot_sprot.fasta.gz
```

When the extraction is complete, you will find the database file in FASTA format in the same directory:

```
ls -lh
```

Output:

```
total 267M
-rw-rw-r-- 1 user user 267M Feb 10 15:00 uniprot_sprot.
↪fasta
```

## 5.5 View the database

Since this extracted database file is large, you can use the head command to view the first few lines of the file:

```
head -n 5 uniprot_sprot.fasta
```

Output:

```
>sp|Q6GZX4|001R_FRG3G Putative transcription factor 001R
↪OS=Frog virus 3 (isolate Goorha) OX=654924 GN=FV3-001R
↪PE=4 SV=1
MAFSAEDVLKEYDRRRRMEALLLSLYYPNDRKLLDYKEWSPPRVQVECPKAPVEWNNPPS
EKGLIVGHFSGIKYKGEKAQASEVDVNKMCCWVSKFKDAMRRYQGIQTCKIPGKVLSDLD
AKIKAYNLTVEGVEGFVRYSRVTKQHVAAFLKELRHSKQYENVNLIHYILTDKRVDIQHL
EKDLVKDFKALVESAHRMRQGHMINVKYILYQLLKKHGHGPDGPDILTVKTGSKGVLYDD
```

Alternatively, you can use the less command to view it one page at a time:

```
less uniprot_sprot.fasta
```

If you would like to count the number of sequences in the database, you can use the `grep` command.

```
grep ">" -c uniprot_sprot.fasta
```

Output:

```
564277
```

The `-c` option of `grep`, counts the number of times the given search string (> in this case) occurs in the input file.

**Note:** A sequence in a FASTA format should start with the > character. Hence, counting the number of times it occurs gives the number of sequences in the file.

You can now proceed towards formatting the database.

## 5.6 Format the database — `makeblastdb`

The database needs to be formatted before it can be used in a BLAST search. You can format it using the `makeblastdb` command, which is part of the NCBI BLAST+ package.

The command has multiple options. Here is an example:

```
makeblastdb -in uniprot_sprot.fasta -parse_seqids \
-title "Swiss-Prot" -dbtype prot -out swissprot
```

**Note:** The \ character splits the long command into multiple lines.

Output:

```
Building a new DB, current time: 03/24/2021 15:12:50
New DB name:   /home/user/databases/swissprot
New DB title:  Swiss-Prot
Sequence type: Protein
Keep MBits: T
Maximum file size: 1000000000B
Adding sequences from FASTA; added 564277 sequences in 47.
↪507 seconds.
```

What the options mean:

**`-in`** File name containing input sequences.

**`-parse_seqids`** Parse sequence identifiers from the input file.

These will be displayed in search results.

**-title** A descriptive name for this database.

**-dbtype** The type of input sequences — acceptable values are `prot` (for protein) and `nucl` (for nucleotide) sequences.

**-out** The value here will be used to name the output files. This is also the name you will need to use for the database while doing a search (see *New DB Name*) in output.

When formatting is complete, you will notice the following files in the `databases` directory:

```
ls -lh
```

Output:

```
total 585M
-rw-rw-r-- 1 user user 100M Mar 24 15:13 swissprot.phr
-rw-rw-r-- 1 user user 4.4M Mar 24 15:13 swissprot.pin
-rw-rw-r-- 1 user user 2.2M Mar 24 15:13 swissprot.pog
-rw-rw-r-- 1 user user  18M Mar 24 15:13 swissprot.psd
-rw-rw-r-- 1 user user 411K Mar 24 15:13 swissprot.psi
-rw-rw-r-- 1 user user 195M Mar 24 15:13 swissprot.psq
-rw-rw-r-- 1 user user 267M Feb 10 15:00 uniprot_sprot.
↪fasta
```

# 6 Search database using query sequence

With the query sequence downloaded and the database downloaded and formatted, you can start performing a BLAST search.

First, change into the directory containing the query sequence:

```
cd ~/projects/sars-cov-2
```

Now run the `blastp` command using the query sequence and the complete path to the database:

```
blastp -query P0DTC2.fasta \
-db /home/user/databases/swissprot \
-out blastp-results.txt \
-outfmt "7 sacc stitle qlen slen pident"
```

What the options mean:

**`-query`** Path to the query sequence.

**`-db`** Complete path of the sequence database.

**`-out`** File to save results to.

**`-outfmt`** Format of the output file. This will use format option 7 (tab-delimited text) and include the following information:

- accession number and description of matching sequences (`sacc` and `stitle`),
- query and subject sequence lengths (`qlen` and `slen`)
- percentage identity of the match (`pident`).

When the database search is complete, you can open `blastp-results.txt` to view the results:

```
less -S blastp-results.txt
```

The `-S` option of the `less` command disables word-wrap.

Output:

```
# BLASTP 2.9.0+
# Query: sp|P0DTC2|SPIKE_SARS2 Spike glycoprotein
↪OS=Severe acute respiratory syndrome coronavirus 2
↪OX=2697049 GN=S PE=1 SV=1
# Database: /home/user/databases/swissprot
# Fields: subject acc., subject title, query length,
↪subject length, % identity
# 88 hits found
P0DTC2      Spike glycoprotein OS=Severe acute
↪respiratory syndrome coronavirus 2 OX=2697049 GN=S PE=1
↪SV=1 1273    1273    100.000
P59594      Spike glycoprotein OS=Severe acute
↪respiratory syndrome coronavirus OX=694009 GN=S PE=1
↪SV=1    1273    1255    76.038
```

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```
Q3LZX1      Spike glycoprotein OS=Bat coronavirus HKU3⍁
↪OX=442736 GN=S PE=3 SV=1     1273    1242    76.041
Q3I5J5      Spike glycoprotein OS=Bat coronavirus Rp3/
↪2004 OX=349344 GN=S PE=1 SV=1 1273    1241    75.334
Q0Q475      Spike glycoprotein OS=Bat coronavirus 279/
↪2005 OX=389167 GN=S PE=3 SV=1 1273    1241    74.745
...
```

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