

Judit Szarvas, postdoc Infectious disease bioinformatics, April 2022

## Data acquisition from public databases for infectious disease studies

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#### Infectious disease often requires context

- Studies frequently require a more global outlook
- Global supply chains make foodborne disease travel long distances
- Initiatives for increased reproducibility and open science prompted scientific publishers and journals to require sequencing data to be openly available at manuscript submission
- Dedicated repositories for sequencing data AND the related metadata



#### **INSDC**

International Nucleotide Sequence Database Collaboration:

– DDBJ (Japan)– DRX/DRR0000001

– EMBL-EBI (Europe)ERX/ERR0000001

NCBI (United States)SRX/SRR0000001

• Data deposited in either system are available from all (after few days)

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



#### **Common data structure**

ENA	NCBI	Description
Study	BioProject	the encompassing research project fx. Citrobacter freundii samples collected from microbiology laboratories of Genovia in 2015
Sample	BioSample	a biological sample fx. swab
Experiment	Experiment	sequencing experiment for a sample and its technical replicates, seq. library and platform/instrument fx. Illumina HiSeq 2000, paired layout, random selection
Run	Run	sequencing run

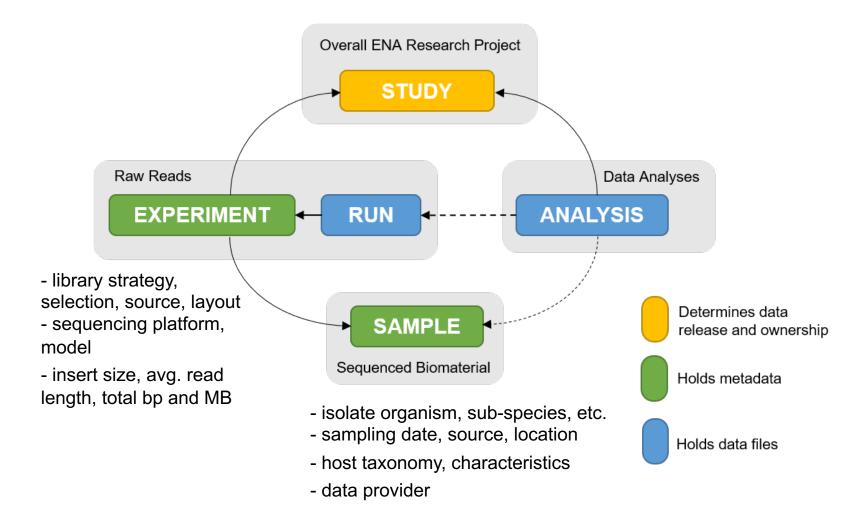
 $\underline{https://www.ncbi.nlm.nih.gov/sra/docs/submitbio/}$ 

https://www.ncbi.nlm.nih.gov/sra/docs/submitportal/

 $\underline{https://ena-docs.readthedocs.io/en/latest/submit/general-guide/metadata.html}$ 



#### ENA metadata model





#### How to cite specific data?

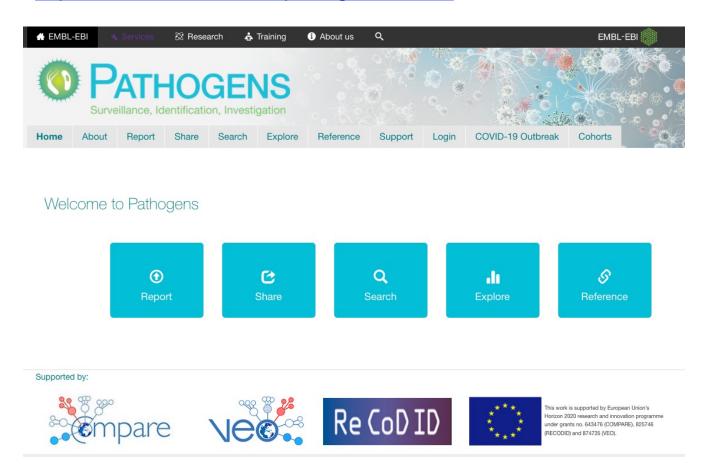
Known identifier*	Cite	
Manuscript DOI and Study/Project accession	Manuscript DOI and Study/Project accession	
Study/Project accession	Study/Project accession	
Record accession	Study/Project accession and individual record accession	

<sup>\*</sup> Do your best to find a manuscript doi



#### **EMBL-EBI ENA - Pathogens**

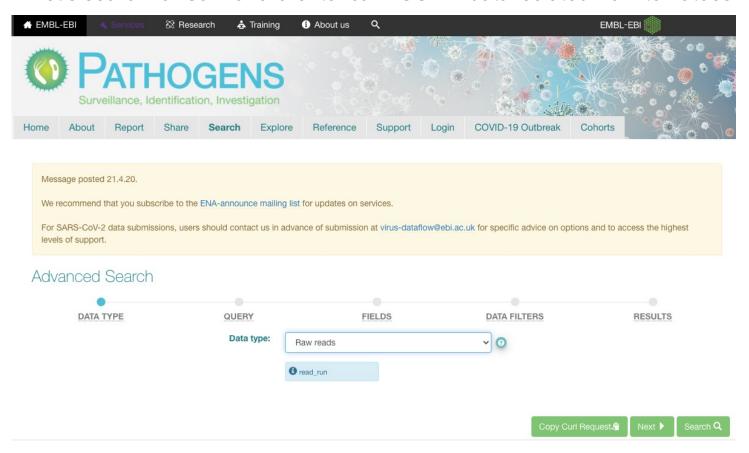
- EMBL-EBI Pathogens is the infectious disease sub-portal of ENA
- Advanced search has indexed sample attributes: https://www.ebi.ac.uk/ena/pathogens/search





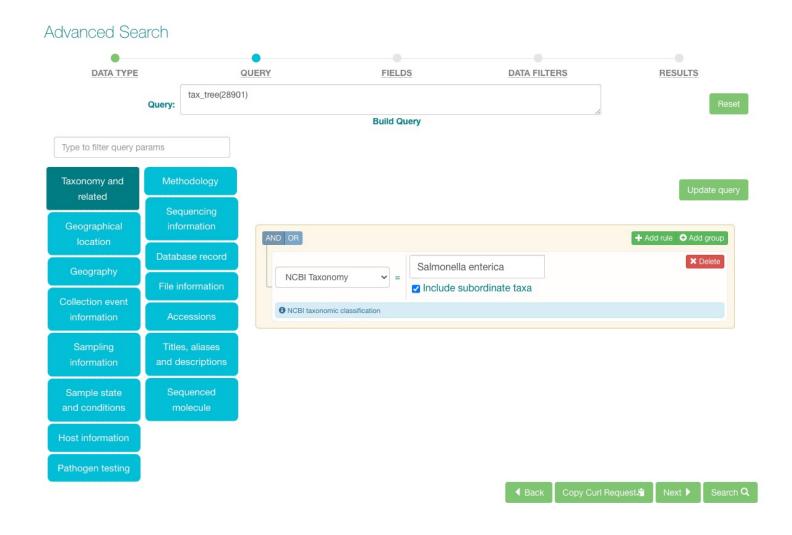
#### Pathogens - Advanced search

- Data type: the type of the data record in search results
- The metadata for the linked samples and experiments are jointly searched!
- Let's search for Salmonella enterica WGS PE data isolated from tomatoes



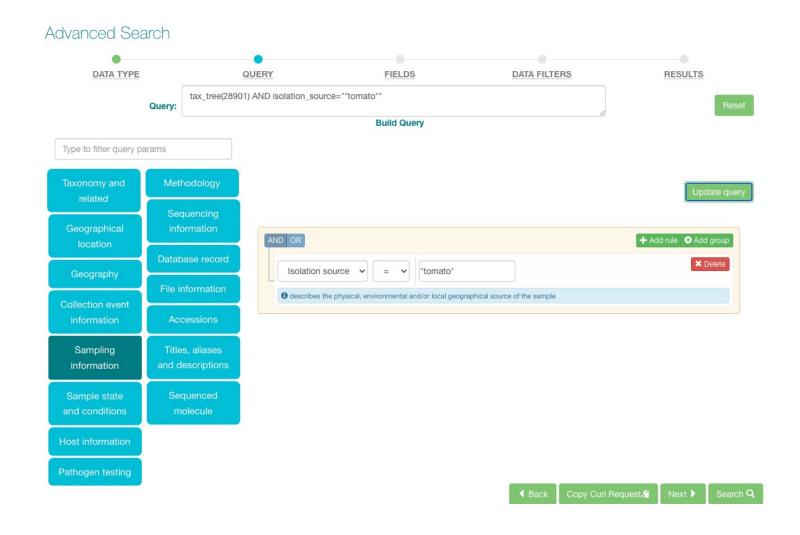


#### Pathogens – Isolate taxonomy



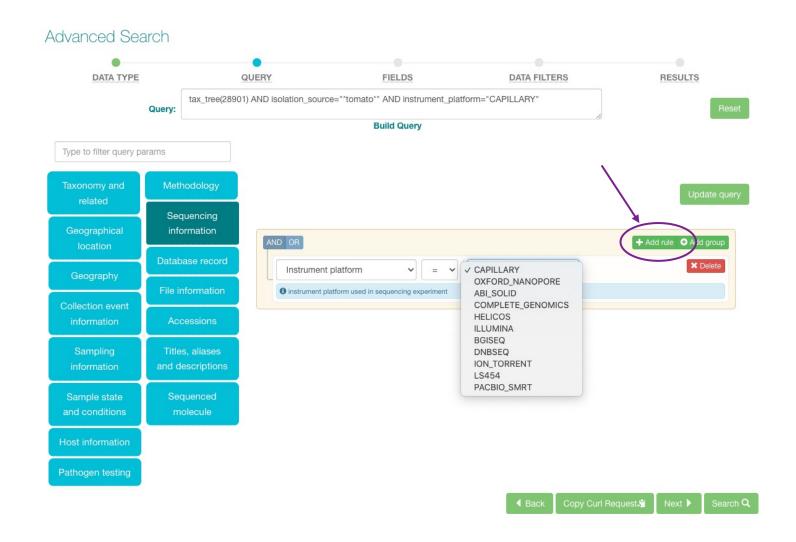


#### Pathogens – Isolate source



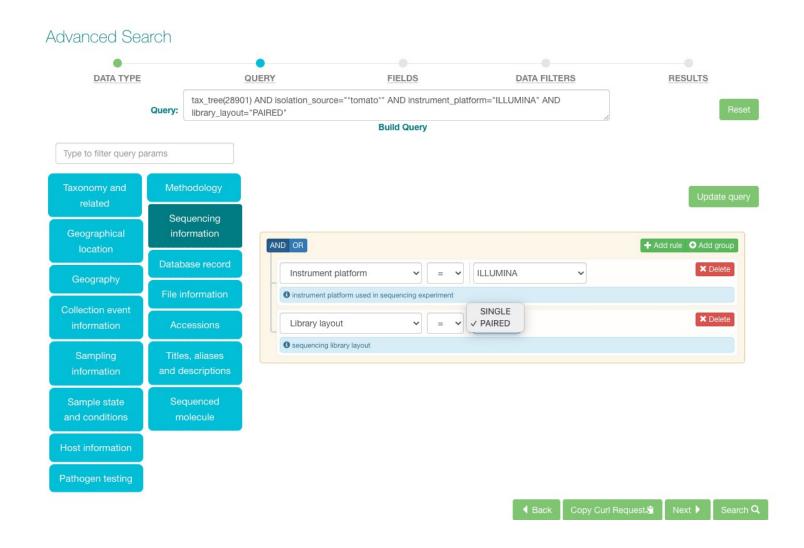
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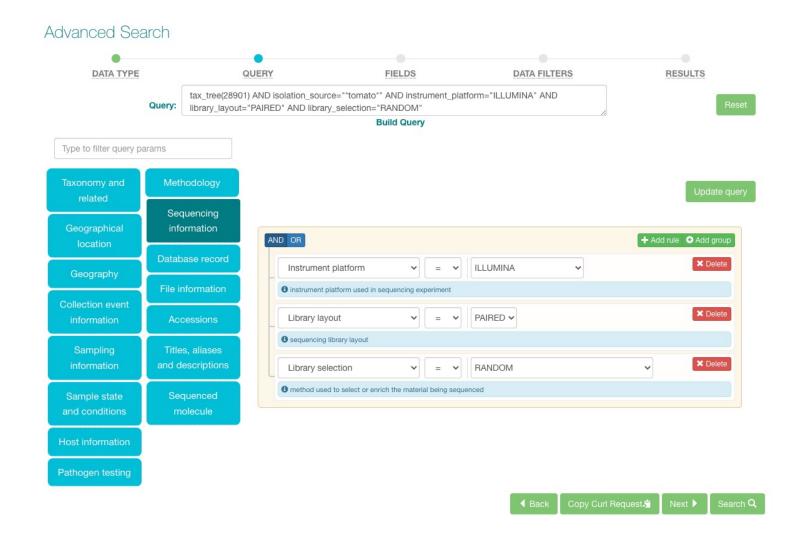
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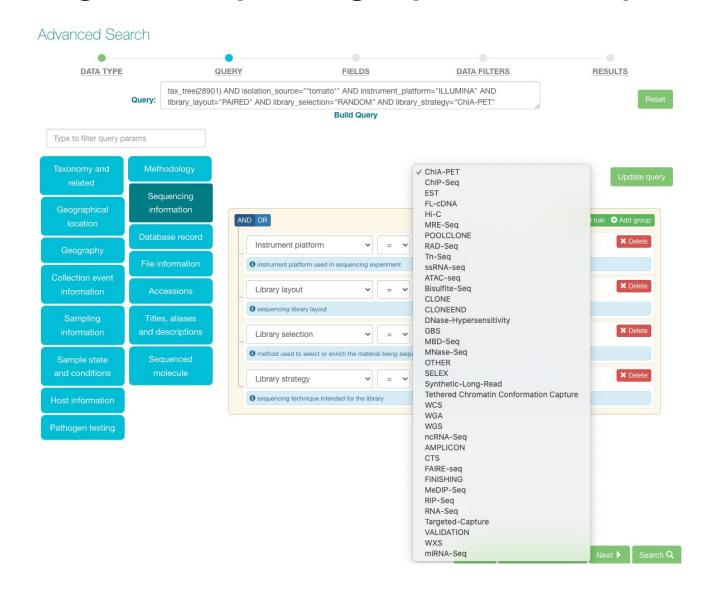
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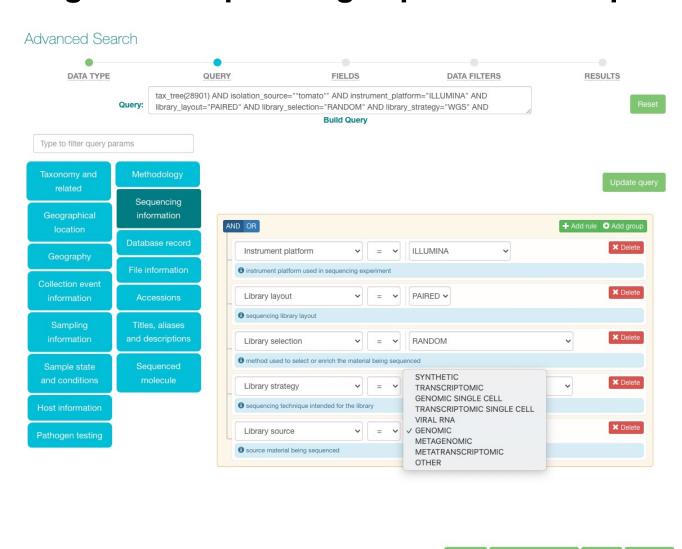
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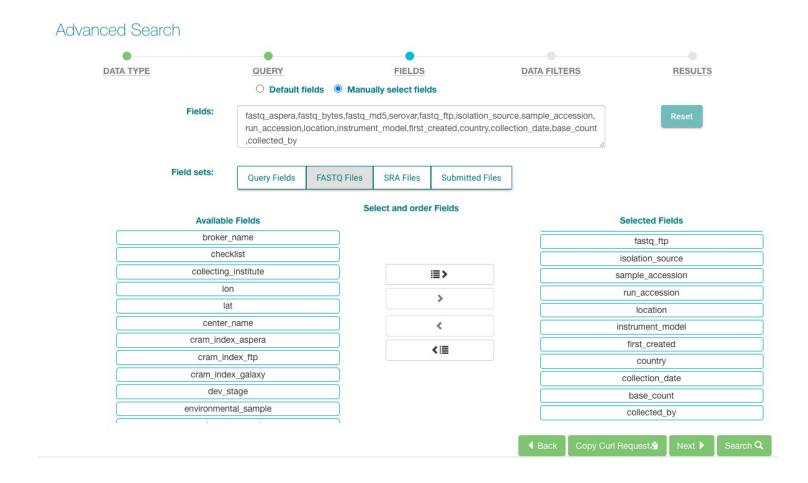




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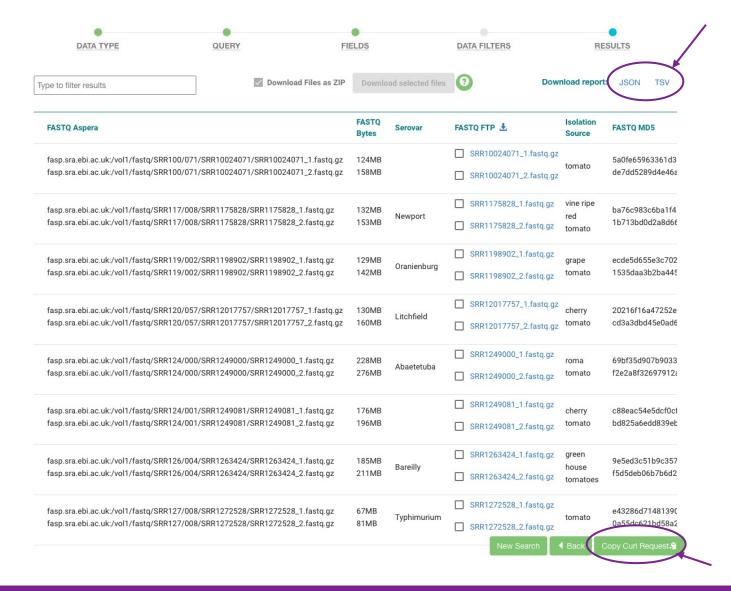
#### Pathogens – Result attributes/fields



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#### Pathogens – Results



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#### Pathogens – Results via curl

```
curl -X POST -H "Content-Type: application/x-www-form-urlencoded" -d "dataPortal=pathogen&result=read_run&query=tax_tree(28901)%20AND%20is olation_source%3D%22*tomato*%22%20AND%20instrument_platform%3D%22ILLU MINA%22%20AND%20library_layout%3D%22PAIRED%22%20AND%20library_selection%3D%22RANDOM%22%20AND%20library_strategy%3D%22WGS%22%20AND%20library_source%3D%22GENOMIC%22&fields=fastq_aspera%2Cfastq_bytes%2Cserovar%2Cfastq_ftp%2Cisolation_source%2Cfastq_md5%2Csample_accession%2Crun_accession%2Clocation%2Clat%2Cinstrument_model%2Cfirst_created%2Ccountry%2Ccollection_date%2Clon%2Cbase_count%2Ccollected_by&limit=0&format=tsv" https://www.ebi.ac.uk/ena/portal/api/search > search_results.tsv
```

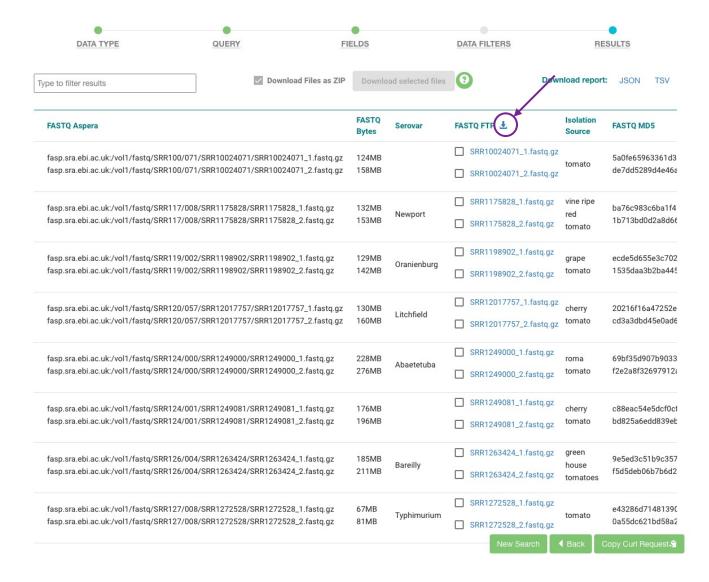
url encoding for special characters:

```
%22 = "
%2C = ,
%3D = :
%20 = space
```

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#### Pathogens – Raw data download via browser



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#### Pathogens – Raw data download via tsv

astq\_aspera fastq\_bytes serovar

fastq\_ftp location isolation source

fastq\_md5

sample\_accession

run accession

instrument model

first\_created country

collection date lon base count collected by

fasp.sra.ebi.ac.uk:/vol1/fastq/SRR100/071/SRR10024071/SRR10024071\_1.fastq.gz;fasp.sra.ebi.ac.uk:/vol1/fastq/SRR100/0 71/SRR10024071/SRR10024071\_2.fastq.gz 129552490;165286840

ftp.sra.ebi.ac.uk/vol1/fastq/SRR100/071/SRR10024071/SRR10024071\_1.fastq.gz;ftp.sra.ebi.ac.uk/vol1/fastq/S

RR100/071/SRR10024071/SRR10024071\_2.fastq.gz tomato

5a0fe65963361d3c933359ca4d5772fa;de7dd5289d4e46a01c23b079f4c3a14e

SAMN12662374

SRR10024071

Illumina MiSeq

2019-09-02 USA:NY

2019-08-09

402393561 NYSDOH

fasp.sra.ebi.ac.uk:/vol1/fastg/SRR117/008/SRR1175828/SRR1175828\_1.fastg.gz;fasp.sra.ebi.ac.uk:/vol1/fastg/SRR117/008/ SRR1175828/SRR1175828\_2.fastq.gz 138525092;160096679 Newport

ftp.sra.ebi.ac.uk/vol1/fastq/SRR117/008/SRR1175828/SRR1175828\_1.fastq.gz;ftp.sra.ebi.ac.uk/vol1/fastq/SRR

117/008/SRR1175828/SRR1175828\_2.fastq.gz vine ripe red tomato

ba76c983c6ba1f41716133d279856aae;1b713bd0d2a8d6663e1b0f22532ebf7b

SAMN02345427

SRR1175828

Illumina MiSeq

2014-03-03 USA:IN

2012-01-03

394193056 FDA

fasp.sra.ebi.ac.uk:/vol1/fastg/SRR119/002/SRR1198902/SRR1198902\_1.fastg.gz;fasp.sra.ebi.ac.uk:/vol1/fastg/SRR119/002/ SRR1198902/SRR1198902\_2.fastq.gz 135077054;149106613 Oranienburg

ftp.sra.ebi.ac.uk/vol1/fastq/SRR119/002/SRR1198902/SRR1198902\_1.fastq.gz;ftp.sra.ebi.ac.uk/vol1/fastq/SRR

119/002/SRR1198902/SRR1198902\_2.fastq.gz

grape tomato

ecde5d655e3c702c80b0f21de5d95a68;1535daa3b2ba44593cd02d6001aa3ad2

SAMN02345583

SRR1198902

Illumina MiSeq

2014-03-20 USA:TX

2012-06-07

367572158 FMA



#### Pathogens – Raw data download with wget

```
wget --quiet --directory-prefix=<download_dir_path> --timeout=30
--tries=3 --waitretry=2
ftp.sra.ebi.ac.uk/vol1/fastq/SRR100/071/SRR10024071/SRR10024071_
1.fastq.gz
```

- --timeout: connection is kept alive until X sec, even if no data is received
- --tries: in case the connection breaks, it attempts to re-connect and <u>continue</u> downloading X times
- --waitretry: X sec of sleep between re-connecting



#### Pathogens – Raw data download in a loop

```
cut -f 4 search_results.tsv | cut -d";" -f 1 > fastq_urls.lst

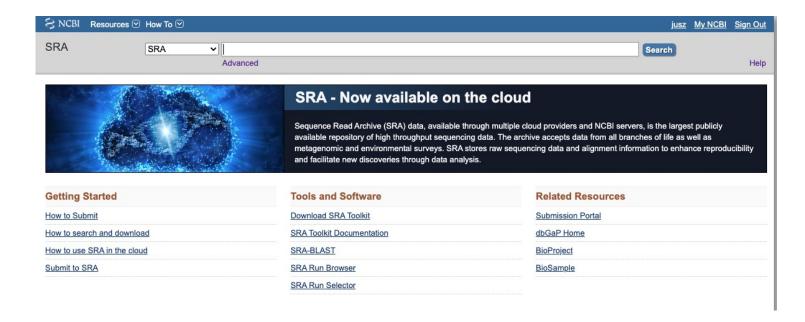
while read FWD_URL;

do
    wget --quiet --directory-prefix=<download_dir_path> --timeout=30
--tries=3 --waitretry=2 ${FWD_URL};
    wget --quiet --directory-prefix=<download_dir_path> --timeout=30
--tries=3 --waitretry=2 ${FWD_URL/_1.fastq.gz/_2.fastq.gz};

done<fastq_urls.lst</pre>
```



#### **NCBI Short Read Archive**



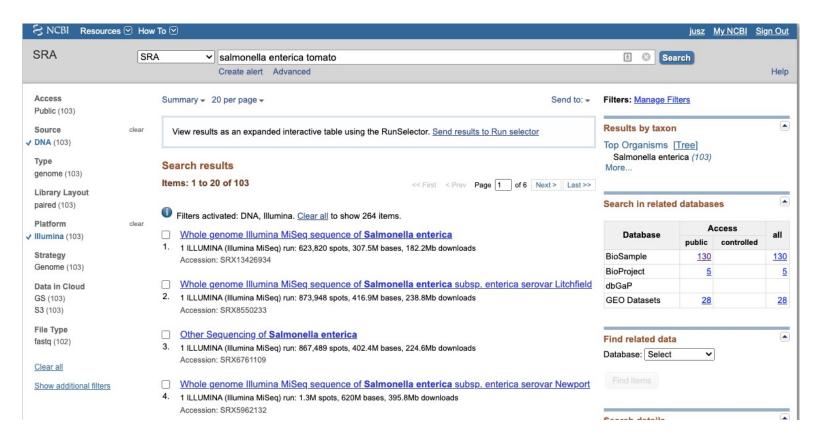
https://www.ncbi.nlm.nih.gov/sra

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#### NCBI SRA search

- A possible workflow:
  - construct the search query by refining the filters via the SRA website <a href="https://www.ncbi.nlm.nih.gov/sra">https://www.ncbi.nlm.nih.gov/sra</a>



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#### NCBI SRA runinfo

 use the Trace SRA API access to download a runinfo csv with run records for this query https://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?save=efetch&db=sra&ret type=runinfo&term=query\_string

```
Run, ReleaseDate, LoadDate, spots, bases, spots with mates, avqLength, size MB, AssemblyName, down
load path, Experiment, LibraryName, LibraryStrategy, LibrarySelection, LibrarySource, LibraryLa
yout, InsertSize, InsertDev, Platform, Model, SRAStudy, BioProject, Study Pubmed id, ProjectID, Sa
mple, BioSample, SampleType, TaxID, ScientificName, SampleName, glk pop code, source, glk analysi
s group, Subject ID, Sex, Disease, Tumor, Affection Status, Analyte Type, Histological Type, Body
Site, CenterName, Submission, dbgap study accession, Consent, RunHash, ReadHash
SRR3476842,2016-05-10 00:00:00,2016-05-05
04:37:42,2278432,455686400,2278432,200,164,,https://sra-downloadb.st-
va.ncbi.nlm.nih.gov/sos2/sra-pub-run-
3/SRR3476842/SRR3476842.1,SRX1741527,63 E4743,WGS,RANDOM,GENOMIC,PAIRED,0,0,ILLUMINA,Illu
mina HiSea
1000, SRP074336, PRJNA320449, ,320449, SRS1421088, SAMN04939497, simple, 90371, Salmonella
enterica subsp. enterica serovar Typhimurium, 63 E4743,,,,,,no,,,,,"INSTITUTE OF CLINICAL
PATHOLOGY AND MEDICAL RESEARCH, WESTMEAD
HOSPITAL", SRA423252, , public, E536B20BF1274C27823BBC050C1FF31B, E9FF0E1D4F3163C3D7369089344C
5B0C
SRR7723982,2018-08-20 16:22:11,2018-08-20
16:15:57,362046,172455250,362046,476,119,,https://sra-downloadb.be-
md.ncbi.nlm.nih.gov/sos3/sra-pub-run-20/SRR7723982/SRR7723982.1,SRX4580494,Nextera XT
library SEQ000077814, WGS, RANDOM, GENOMIC, PAIRED, 500, 0, ILLUMINA, Illumina
MiSeq,SRP018785,PRJNA186035,2,186035,SRS3694824,SAMN09865679,simple,108619,Salmonella
enterica subsp. enterica serovar
Newport, CFSAN001892,,,,,,no,,,,,CFSAN, SRA761059,,public,FC70D7DAD3ED37217A12F4F3925509FF
,4CE6BFBF4C84E4E484EBA60C35032196
```



#### NCBI SRA sample metadata

 use SRA API to download biosample metadata for individual runs https://www.ncbi.nlm.nih.gov/sra/?term=SRR6666614&format=text

```
Accession: SRX3643475
Title: Whole genome Illumina MiSeq sequence of Salmonella enterica
Experiment Design: MiSeq deep shotgun sequencing of cultured isolate.
Submission: CFSAN
Study accession: SRP098999
Study Title: GenomeTrakr Project: Michigan Department of Agriculture and Rural Development
Study Abstract: Whole genome sequencing of cultured Salmonella enterica as part of the US Food and Drug
Administration's WGS surveillance effort for the rapid traceback of foodborne pathogens.
Study Center:
Study Center Project: Salmonella enterica (ID=)
Project name: Salmonella enterica
Sample Accession: SRS2908290
Sample Description:
Sample Common Name: , (TaxonId=28901)
Sample Attributes: collection date=2018; isolation source=cherry tomato; source type=Food; IFSAC+
Category=seeded vegetables (solanaceous); ontological term=miniature tomato (whole,
raw):FOODON 03311800; attribute package=environmental/food/other; strain=18FMFA000068-10;
collected by=Michigan Department of Agriculture and Rural Development; lat lon=missing;
qeo loc name=USA:MI; isolate name alias=CFSAN074863; PublicAccession=CFSAN074863;
ProjectAccession=PRJNA368989; Species=enterica; Genus=Salmonella; BioSampleModel=Pathogen.env
Sample Links: =
Library Name: Nextera XT library SEQ000068723
Library Strategy: WGS
Library Source: GENOMIC
Library Selection: RANDOM
Library Layout: PAIRED, Orientation: , Nominal length: 500, Nominal Std Dev:
Platform Name: ILLUMINA
Spot descriptor: 1) Application Read, Forward; 2) Application Read, Reverse
Total: 1 run, 2M spots, 694.3M bases
Run #1: SRR6666614, 2022210 spots, 694252924 bases
```



#### NCBI SRA run download

- use NCBI SRA Toolkit prefetch and fasterq-dump to download raw reads using their run accession
  - installed on computerome: sratoolkit/3.0.0
  - for downloading run (fastq) files
     <a href="https://github.com/ncbi/sra-tools/wiki/08.-prefetch-and-fasterq-dump">https://github.com/ncbi/sra-tools/wiki/08.-prefetch-and-fasterq-dump</a>
  - it downloads the .sra file, which is a "compressed" file format, and fasterq-dump is needed to unpack it (uses CPU time!):

```
prefetch SRR3476842;
fasterg-dump SRR3476842;
```



#### **NCBI SRA** programmatically

- NCBI Entrez utilities
  - for programmatic access<a href="https://www.ncbi.nlm.nih.gov/books/NBK25499/">https://www.ncbi.nlm.nih.gov/books/NBK25499/</a>
  - recommended to use a parser, for example biopython's Entrez module:
     https://biopython.org/docs/latest/api/Bio.Entrez.html



### **Exercise**

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#### **Exercise**

Search in ENA Pathogens for *Listeria monocytogenes* isolates that were in connection with sprouts (any kind), sampled after 2015-01-01, and shotgun whole-genome sequenced on an Illumina platform.

Gather ftp download paths and metadata regarding collection date, country and source, together with accession numbers for the run, sample, and study.

Download one (1) sample from the most recent ones using either wget (you can run on the login node) or sratoolkit (create and submit a job for it)