Data acquisition and data management

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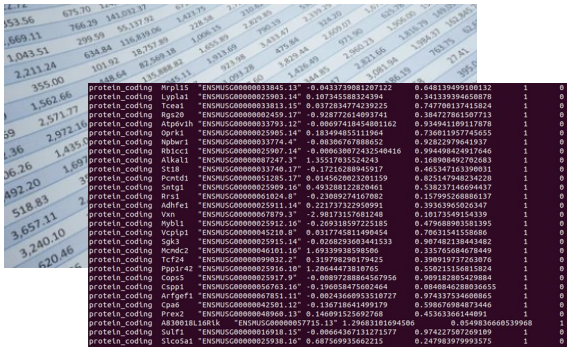
We are going to use the interactive Q&A tool Mentimeter, please connect here (from your phone or computer):

https://www.menti.com

[CODE:]

[GABIO_20240416_Data , 2 slides]

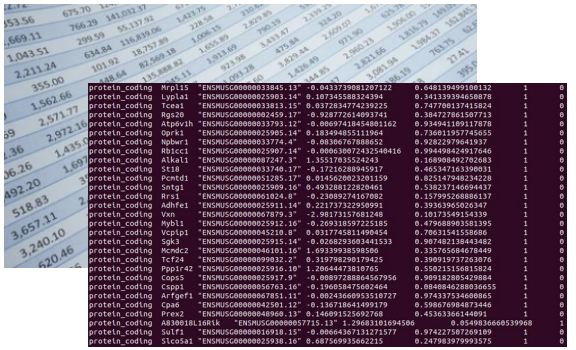
Before data analysis:



Shutterstock thumbnails

Curate lab notes and acquired data into a structured format that is computer-readable.

Before data analysis:



Shutterstock thumbnails

Curate lab notes and acquired data into a structured format that is computer-readable.

After data acquisition and analysis:

ENA European Nucleotide Archive

ARTIC NETWORK ARTICnetwork

NIH National Library of Medicine National Center for Biotechnology Information
Sequence Read Archive Search Run Browser Analyses

ProteomeXchange

EUROPEAN GENOME-PHENOME ARCHIVE

NIH NATIONAL CANCER INSTITUTE GDC Data Submission Portal

GEO Gene Expression Omnibus

FLOWRepository

Publication and FAIR sharing of well-annotated data with the scientific community.

Outline

0. Before the experiment: Collect samples and sample metadata.

1. What data do I have?

2. What information do I want to extract?

3. How to search for software tools that suit my needs?

4. How do I know whether a software tool is a good choice - before even downloading it?

5. How to make my data “AI-compatible”.

6. After data analysis: Publication and FAIR sharing of data.

0. Before the experiment: Collect samples and sample metadata.

What is your area of research?

https://www.menti.com

[CODE:]

[GABIO_Data_20240416]

0. Before the experiment: Collect samples and sample metadata.

Which technologies do you use to generate data?

https://www.menti.com

[CODE:]

[GABIO_Data_20240416]

0. Before the experiment: Collect samples and sample metadata.

Use one sample identifier throughout all steps of the experiment.

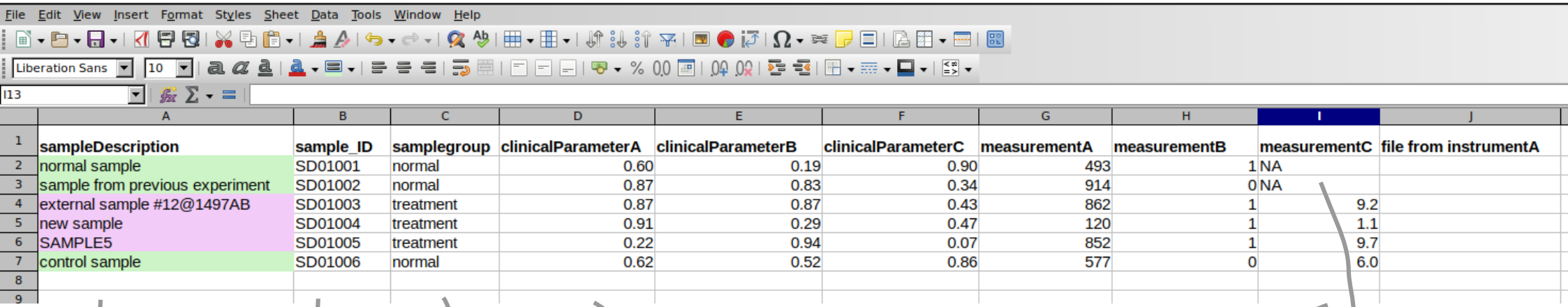
Use the same metadata grid for all samples in the experiment.

Label your samples carefully and in a meaningful and systematic way.

0. Before the experiment: Collect samples and sample metadata.

- Which samples do I have? Do I have several sample groups (experimental conditions…)?
- What are the parameters I will measure for my samples?
- Which instrument(s) will be used for the measurements?

0. Before the experiment: Collect samples and sample metadata.



	A	B	C	D	E	F	G	H	I	J
1	**sampleDescription**	**sample_ID**	**samplegroup**	**clinicalParameterA**	**clinicalParameterB**	**clinicalParameterC**	**measurementA**	**measurementB**	**measurementC**	**file from instrumentA**
2	normal sample	SD01001	normal	0.60	0.19	0.90	493	1	NA	
3	sample from previous experiment	SD01002	normal	0.87	0.83	0.34	914	0	NA	
4	external sample #12@1497AB	SD01003	treatment	0.87	0.87	0.43	862	1	9.2	
5	new sample	SD01004	treatment	0.91	0.29	0.47	120	1	1.1	
6	SAMPLE5	SD01005	treatment	0.22	0.94	0.07	852	1	9.7	
7	control sample	SD01006	normal	0.62	0.52	0.86	577	0	6.0	
8										
9										

free text sample description (incl. different colours, fonts)

structured sample identifier

structured samplegroup label

existing data

missing data

1. What data do I have?

Know which files are generated, how big these files are
and how to transfer and store them.

Keep raw data well-labelled for long-term storage or later publication.

1. What data do I have?

- Which files are generated on the acquisition instrument computer for each sample?
- Where are these files generated on the instrument computer?
- Are the data in a standard format or in tabular format?
- How big are these data in terms of computer storage?
- For how many samples do I usually generate data in an experiment?

1. What data do I have?

Illumina NextSeq2000 sequencing system

https://dna.uga.edu

Example:
Illumina sequencing data acquisition for genomic variant analysis

- Illumina format raw sequencing output per flowcell
- “sampleSheet.csv” in Illumina format

1. What data do I have?

- Illumina format raw sequencing output per flowcell

> Genomics_data > Illumina_NextSeq > NextSeq-Backup > 211004_NB501433_0169_AHTVNMBGXC

ion ▾ | Tools ▾ | Settings

Name	Size	File Type	Modified Date
Config		Folder	2021-10-04 15:4...
Data		Folder	2021-10-04 19:0...
Images		Folder	2021-10-04 18:1...
InstrumentAnalyticsLogs		Folder	2021-10-05 22:1...
InterOp		Folder	2021-10-05 22:1...
Logs		Folder	2021-10-05 22:1...
Recipe		Folder	2021-10-04 15:4...
RTALogs		Folder	2021-10-05 22:1...
CopyComplete.txt	0 bytes	TXT File	2021-10-05 22:1...
RTAComplete.txt	49 bytes	TXT File	2021-10-05 22:1...
RTAConfiguration.xml	6.1 KB	XML File	2021-10-04 15:5...
RTARead1Complete.txt	36 bytes	TXT File	2021-10-05 05:5...
RTARead2Complete.txt	36 bytes	TXT File	2021-10-05 08:2...
RTARead3Complete.txt	36 bytes	TXT File	2021-10-05 09:1...
RTARead4Complete.txt	37 bytes	TXT File	2021-10-05 22:1...
RunCompletionStatus.xml	927 bytes	XML File	2021-10-05 22:1...
RunInfo.xml	28 KB	XML File	2021-10-04 15:4...
RunParameters.xml	26.3 KB	XML File	2021-10-04 15:4...
SampleSheet.csv	841 bytes	CSV File	2021-10-04 15:4...

Illumina NextSeq2000 sequencing system

https://dna.uga.edu

~ 90 GB
Do not extract or delete subdirectories!

1. What data do I have?

- “sampleSheet.csv” in Illumina format

```
[Header]
Date,20211006
ExperimentName,Jeitler_20211006
Workflow,GenerateFastQ

[Reads]
150
150

[Settings]
InstrumentType,NextSeq
Adapter,AGATCGGAAGAGCACACGTCTGAACTCCAGTCA
AdapterRead2,AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

[Data]
Sample_ID,Sample_Name,I5_Index_ID,index2,I7_Index_ID,index
MJ04001,MJ04001,506,TAAGATTA,701,ATTACTCG
MJ04002,MJ04002,506,TAAGATTA,702,TCCGGAGA
MJ04003,MJ04003,506,TAAGATTA,703,CGCTCATT
MJ04004,MJ04004,506,TAAGATTA,704,GAGATTCC
MJ04005,MJ04005,506,TAAGATTA,705,ATTCAGAA
MJ04006,MJ04006,506,TAAGATTA,706,GAATTCGT
```

Illumina NextSeq2000 sequencing system

https://dna.uga.edu

2. What information do I want to extract?

Store raw data as backup and for publication.

Convert proprietary formats into portable standard or tabular formats for downstream analysis.

2. What information do I want to extract?

Example: a Core Facility

- Which files should be stored as long-term backup?
- Which files are transferred to the users?
- Which files can be shared with Tech Support?

2. What information do I want to extract?

Example: a Core Facility

- Which files should be stored as long-term backup?
- Which files are transferred to the users?
- Which files can be shared with Tech Support?

Example: Core Facility users ~ researchers

- Which files can I use directly for analysis?
- Which files can I use to extract data formated for downstream analysis?
- Which files do I need to deposit in public repositories upon publication?

2. What information do I want to extract?

Do I have to convert raw data on the instrument into a **portable or standard format** that is readable on another computer without specialized software?

File standard for next generation sequencing data (“reads”): **.fastq** format:


```
@M02453:34:000000000-KRWV5:1:1102:14370:9938 2:N:0:320
GGACAGGTAGGACCTGATTTCCTTACTGCCTCTTGCTTCTCTTTTCCTATCCTGAGTAGTGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTT
CCAGGACCAGAAGCCGGGCCAGAGACCAAATGGCACCTTTATCCCCCGACTCAACTTTAATCACACTCCCATCCCTCTCACCCCGACAGACCACACC
+
CCCCCGGGGGGGGFGCDGGGGGGGGGGGGGGGGGGDGGGFGGGGGGGGFFGGGGGEGFEFGFFGFGGGGGGGCEFGGGGGGGGGGFBFGFG>C7EGEGC
(0652/5(/,(((((,(,3((/(((/((((-.51,)(,)))))))(4(((-(4(())-.).4)3.(2((((.(2:)(7,)-((((((4(,2(,(.((
@M02453:34:000000000-KRWV5:1:1102:17992:18799 2:N:0:320
AGACGACAGGGCTGGTGACATCCATGTCACAGAGCTGCGCTGAGGCTAAAGCTGCGACCCTGAAAGCCCCACCCCAGTGACTAGCAGCAGACAGGGCCCG
GCACGGTTTGACAGCAATTTGTTCCCTGGACCGCGTGTTACACCCACAGCTACTATTTGTACATCGTGCGGTCCTAACAGCAGACATATACACGAAC
+
CCCCC7:6@,@;FGGF,;C,;CF,EFGD9FF<,CFE<C@CC:C6D,EFCB@5FG,4=FFFA<,55AF8,4:BF7+F:9<:F;C88A98,94F,DF>B?++
445(31(4(,.))).(.5))))()).))(.:(,,---((..-2(,((((())))).).).)))))(-((-(,((.)),))..((,())).).)(,(,
@M02453:34:000000000-KRWV5:1:1105:16877:10537 2:N:0:320
TGCTTGCCACAGGTCTCCCAAGGCGCACTGGCCTCATCTTGGGCCTGTGTTATCTCCTAGGTTGGCTCTGACTGTACCACCATCCACTACAACTACATG
TGCTGTTCCCCAGCCCCTGCTTGCCTCCGACCTGTCTCTTATCCCCCCCCGACGCTGCCCACCACCACGCCACGCAAAAATACAGTATGTCGCCGCC
+
CCCCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGDGGGGGGGGGGGGGGGFGGGGGGGGFGGGGGGGF
E7=4>8*ACFF)69B(5(-/./*(0/8((,7:4()6)75.).)1),((,-((((,((-4:.86(8(,((((-(4((-4.3().).)))).).--***
@M02453:34:000000000-KRWV5:1:1118:21532:23865 2:N:0:320
GGACAGGTAGGACCTGATTTCCTTACTGCCTCTTGCTTCTCTTTTCCTATCCTGAGTAGTGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTT
GAAGGACAAGAAGCGGAGGGGGAGACCAAGGGGGCAAGTAGGACGCAGAGTCAACTCTAACGACTTTCCATACTCACATAACTCCCATCACCCAAAA
+
CCCCCGGGGGGGGGGFGGGGGGGGGGGGGG8EFGGGGGGGGGGGGGGDFGGGGGGGGGGGGGGGGGFGGGGGGGGEGGGGGGGGGGGGGDGEGGG
5(((873(((/84(,((,((111((((.(((,340((44))),.((,(((.64)))-)))((((-)))-.6()).(.)))(0--/--)1*.)0<))
```


https://dna.uga.edu

Illumina NextSeq2000 sequencing system

- Ensures compatibility between instrument output and data analysis tools
- Allows analysis tool developers to rely on a standard data input format

Linux R python

Linux command-line tools for generation of .fastq files from Illumina raw data: bcl2fastq or BCL convert (Illumina)

2. What information do I want to extract?

Genomic variants:

.fastq files can be further analysed with numerous community-developed and commercial analysis tools.

In the analysis workflow to detect genomic variants, the sequences in .fastq format are first aligned to the reference genome of the organism of origin:

Linux command-line tools for alignment ro the genome: e.g. bwa (https://bio-bwa.sourceforge.net)

2. What information do I want to extract?

From the alignments, genomic variants are extracted and stored in **.vcf** = Variant Call Format:

1 The VCF specification

VCF is a text file format (most likely stored in a compressed manner). It contains meta-information lines, a header line, and then data lines each containing information about a position in the genome. The format also has the ability to contain genotype information on samples for each position.

1.1 An example

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF    ALT     QUAL FILTER INFO                              FORMAT      NA00001        NA00002        NA00003
20     14370   rs6054257 G      A       29   PASS   NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51 1/1:43:5:.,.
20     17330   .         T      A       3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3   0/0:41:3
20     1110696 rs6040355 A      G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2   2/2:35:4
20     1230237 .         T      .       47   PASS   NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 0/0:61:2
20     1234567 microsat1 GTC    G,GTCT  50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4       0/2:17:2       1/1:40:3
```

Linux command-line tools for variant calling: e.g. GATK (https://gatk.broadinstitute.org/hc/en-us)

3. How to search for software tools that suit my needs?

Be aware of the type of data and scientific question at hand.

Find out what are established software tools and workflows in the community.

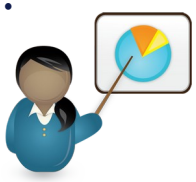
When choosing an analysis workflow from a paper, make sure their and my data and research conditions are comparable.

3. How to search for software tools that suit my needs?

- Will I use the software myself or will I have help with data analysis?
- Will the software work on my operating system?
- Is the software license-dependent or open access?
- Is the software compatible with my type of data?
- Was the software developed with a similar research question in mind?
- Take note from publications and talks on what other researchers in the field use, look out for review/benchmarking articles
- Look up repositories of open source software packages

3. How to search for software tools that suit my needs?

- Tools mentioned at conferences and in publications:



PubMed®

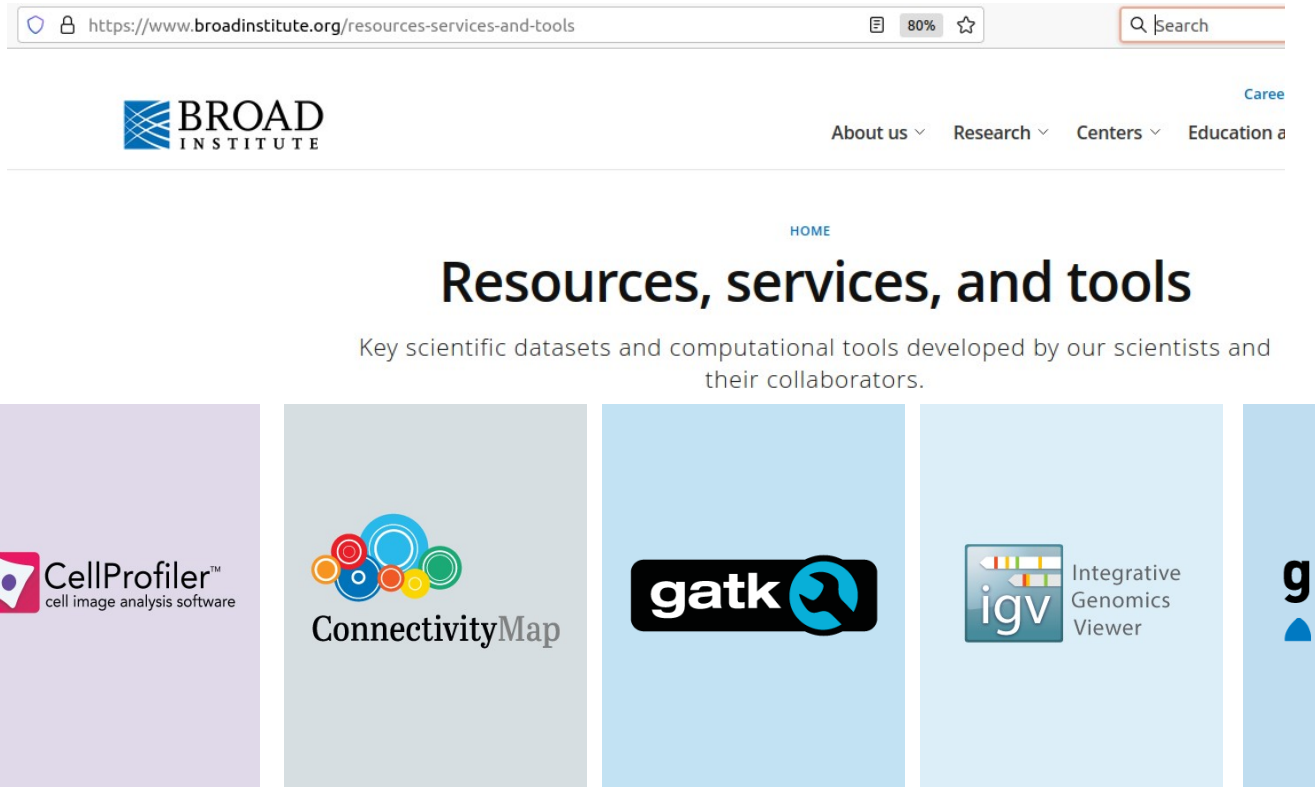
- Repositories of open source software packages:

R

python™

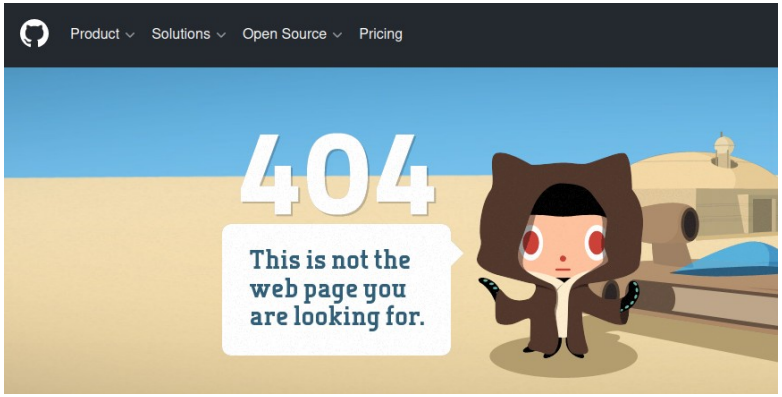
ubuntu

- Institutes that develop many frequently used computational tools:



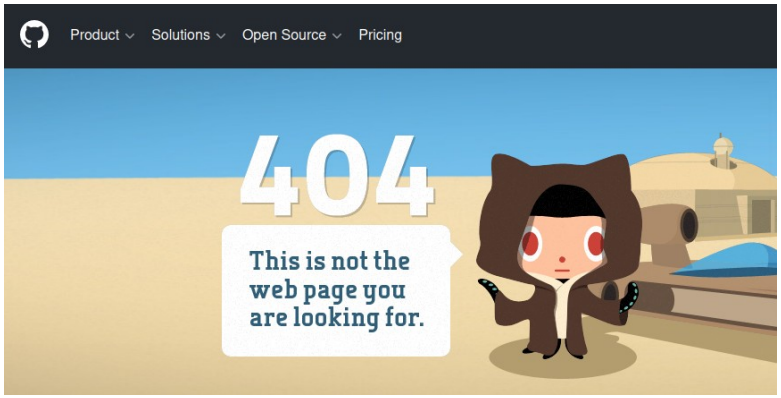
. . .

4. How do I know whether a software tool is a good choice - before even downloading it?



Do not waste time with ill-documented or discontinued computational tools.

4. How do I know whether a software tool is a good choice - before even downloading it?



- Is the download page available (for open source tools) when linking from a publication?
- On the download page, when was the latest update?
- Is there documentation and user guide available? Does the user guide include example use cases? Do these use cases fit my needs?
- Find out from the documentation whether the tool will work with the data format I have as an input.
- Is there a way to contact the developers/tech support of the tool by email?

5. How to make my data “AI-compatible”

Sample data and metadata in a spreadsheet:

	A	B	C	H	I	J
1	**sample_ID**	**samplegroup**	**clinicalParameterA**	**measurement**	**.fastq file**	**.vcf file**
2	SD01001	normal	0.97	NA	/my/filesystem/project1/fastq/SD01001.fastq.gz	/my/filesystem/project1/vcf/SD01001.vcf.gz
3	SD01002	normal	0.84	NA	/my/filesystem/project1/fastq/SD01002.fastq.gz	/my/filesystem/project1/vcf/SD01002.vcf.gz
4	SD01003	treatment	0.88	7.7	/my/filesystem/project1/fastq/SD01003.fastq.gz	/my/filesystem/project1/vcf/SD01003.vcf.gz
5	SD01004	treatment	0.04	5.1	/my/filesystem/project1/fastq/SD01004.fastq.gz	/my/filesystem/project1/vcf/SD01004.vcf.gz
6	SD01005	treatment	0.15	9.5	/my/filesystem/project1/fastq/SD01005.fastq.gz	/my/filesystem/project1/vcf/SD01005.vcf.gz
7	SD01006	normal	0.15	2.8	/my/filesystem/project1/fastq/SD01006.fastq.gz	/my/filesystem/project1/vcf/SD01006.vcf.gz

[. . .]

Or in .csv plain text:

```
sample_ID,samplegroup,clinicalParameterA,,,,measurementC,.fastq file,.vcf file
SD01001,normal,0.97,,,,,NA,/my/filesystem/project1/fastq/SD01001.fastq.gz,/my/filesystem/project1/vcf/SD01001.vcf.gz
SD01002,normal,0.84,,,,,NA,/my/filesystem/project1/fastq/SD01002.fastq.gz,/my/filesystem/project1/vcf/SD01002.vcf.gz
SD01003,treatment,0.88,,,,,7.7,/my/filesystem/project1/fastq/SD01003.fastq.gz,/my/filesystem/project1/vcf/SD01003.vcf.gz
SD01004,treatment,0.04,,,,,5.1,/my/filesystem/project1/fastq/SD01004.fastq.gz,/my/filesystem/project1/vcf/SD01004.vcf.gz
SD01005,treatment,0.15,,,,,9.5,/my/filesystem/project1/fastq/SD01005.fastq.gz,/my/filesystem/project1/vcf/SD01005.vcf.gz
SD01006,normal,0.15,,,,,2.8,/my/filesystem/project1/fastq/SD01006.fastq.gz,/my/filesystem/project1/vcf/SD01006.vcf.gz
```

Your analysis here!

6. After data analysis: Publication and FAIR sharing of data.

Everyone can benefit from well-annotated public datasets (not only computational biologists and data analysts!).

6. After data analysis: Publication and FAIR sharing of data.

Findability:

unique dataset identifier, in a searchable resource, with rich metadata

Accessibility:

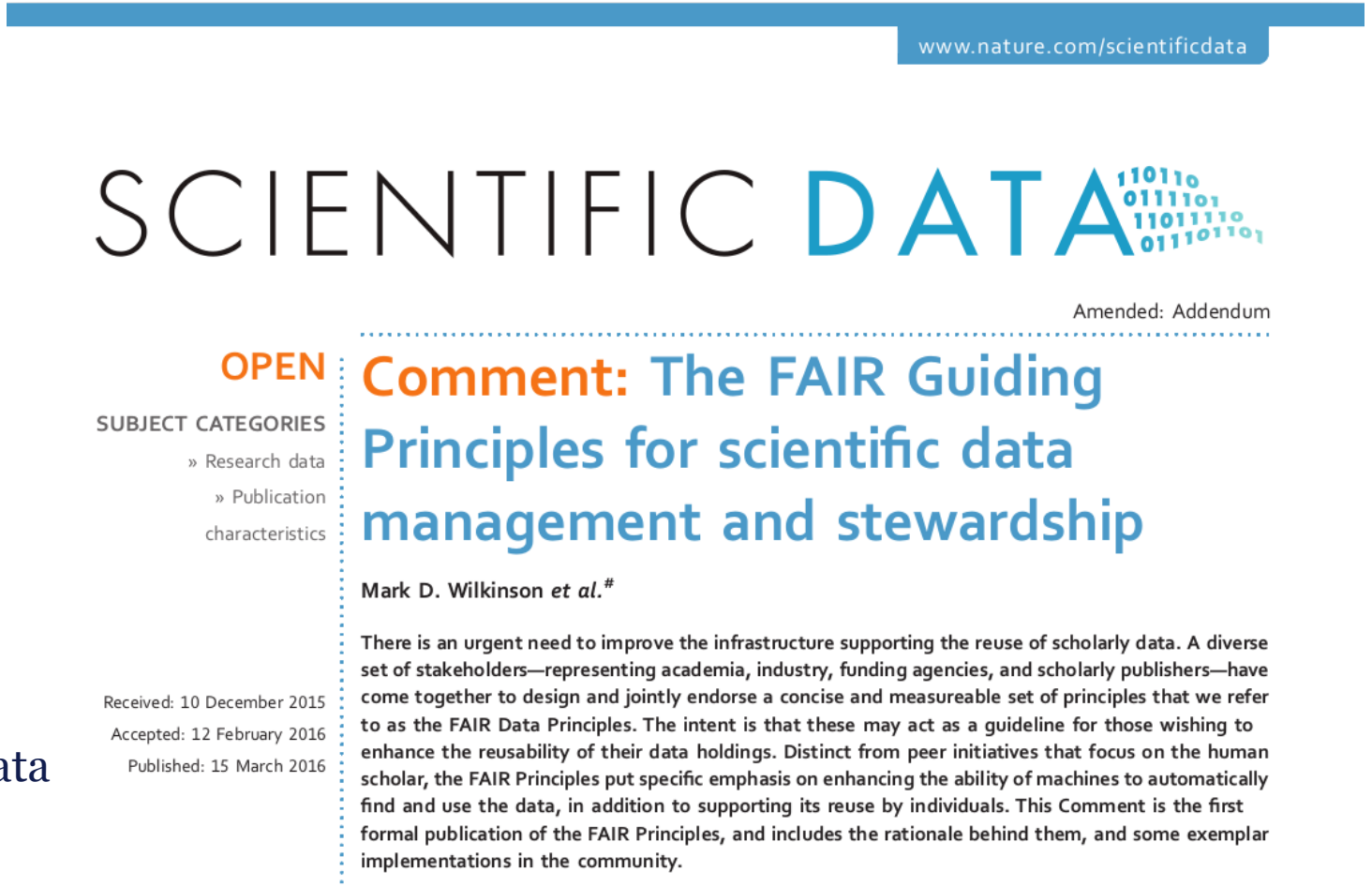
retrievable using a standardized communications protocol, when necessary with authentication

Interoperability

in “standardized” formats, metadata use controlled vocabulary

Reusability

detailed metadata and explicit about authors and data usage license



www.nature.com/scientificdata

SCIENTIFIC DATA

Amended: Addendum

OPEN

SUBJECT CATEGORIES

» Research data

» Publication characteristics

Received: 10 December 2015
Accepted: 12 February 2016
Published: 15 March 2016

Comment: The FAIR Guiding Principles for scientific data management and stewardship

Mark D. Wilkinson *et al.*#

There is an urgent need to improve the infrastructure supporting the reuse of scholarly data. A diverse set of stakeholders—representing academia, industry, funding agencies, and scholarly publishers—have come together to design and jointly endorse a concise and measureable set of principles that we refer to as the FAIR Data Principles. The intent is that these may act as a guideline for those wishing to enhance the reusability of their data holdings. Distinct from peer initiatives that focus on the human scholar, the FAIR Principles put specific emphasis on enhancing the ability of machines to automatically find and use the data, in addition to supporting its reuse by individuals. This Comment is the first formal publication of the FAIR Principles, and includes the rationale behind them, and some exemplar implementations in the community.

6. After data analysis: Publication and FAIR sharing of data.

Which public data repositories have you used or heard about?

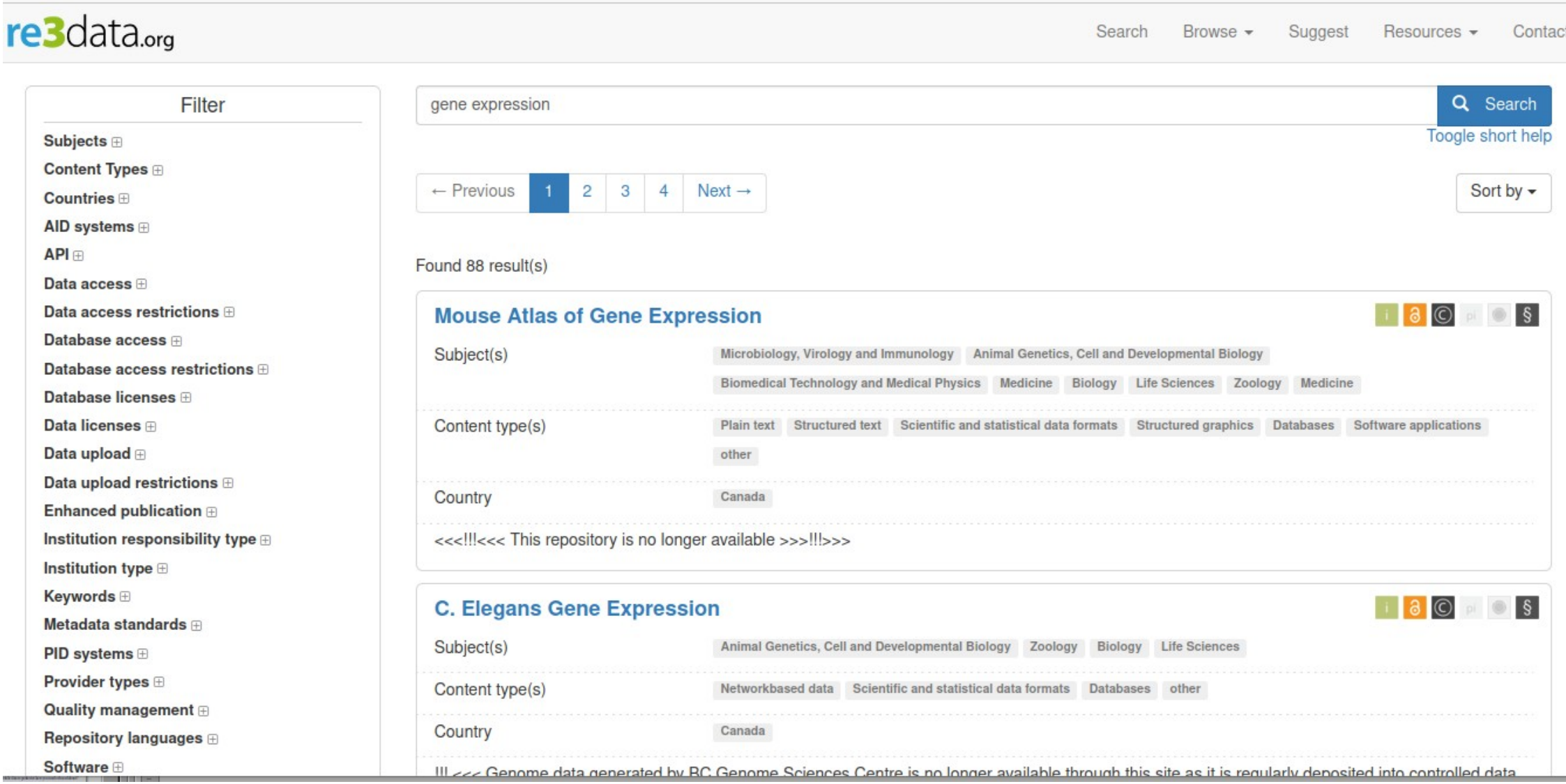
https://www.menti.com

[CODE:]

[GABIO_20240416_Data , last slide]

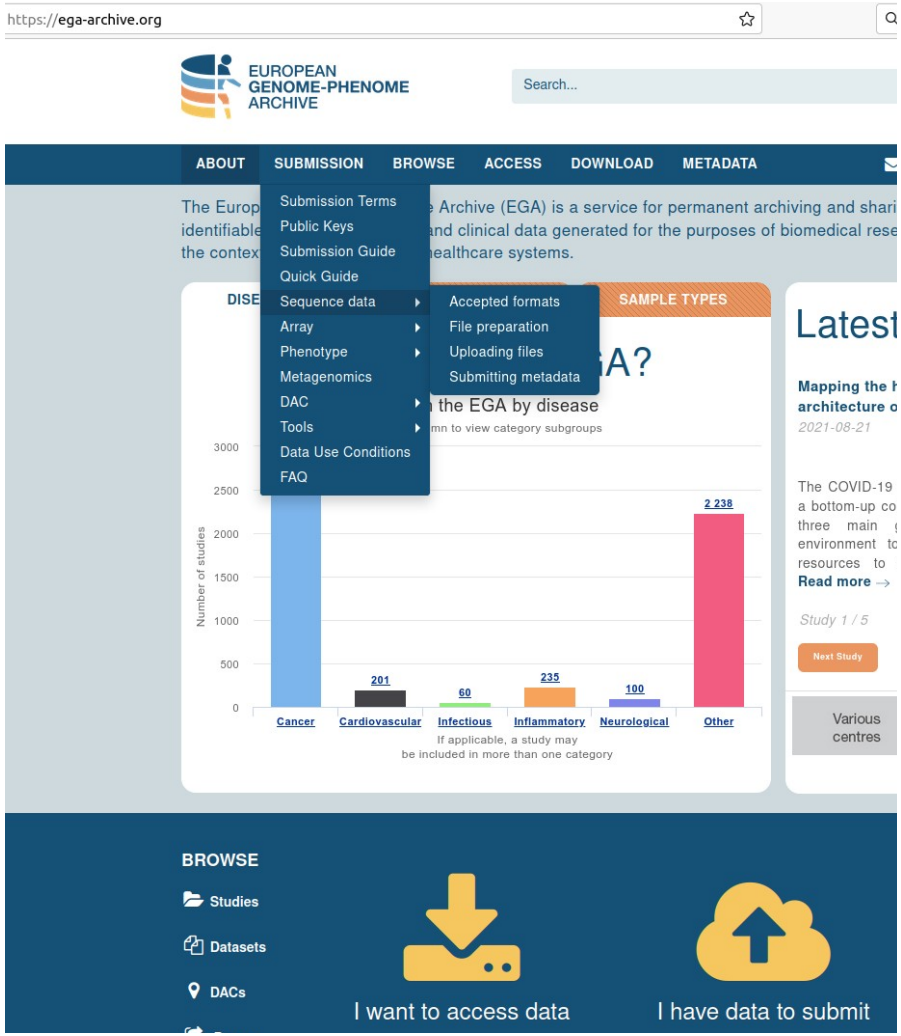
6. After data analysis: Publication and FAIR sharing of data.

re3data.org
REGISTRY OF RESEARCH DATA REPOSITORIES



6. After data analysis: Publication and FAIR sharing of data.

You deposit data:



Get your submission account

Fill the **submission form** and provide details of the data type(s), used platform(s) and estimated size of your submission.
If you are affiliated to an existing consortium, such as the International Cancer Genome Project (ICGC), please add this information in the comments.

Register Study/DAC

Use **Submitter Portal** to register your study, samples, Data Access Committee (DAC) and Policy.

Upload data

Encrypt your data files using the **EgaCryptor** or **encrypt your files localy** and upload it using default FTP clients or Aspera.

Register experiments and runs

Associate each data file to a registered sample and study by **Linking files to samples** . Details of the experimental procedure you followed must be provided.

Finish your submission

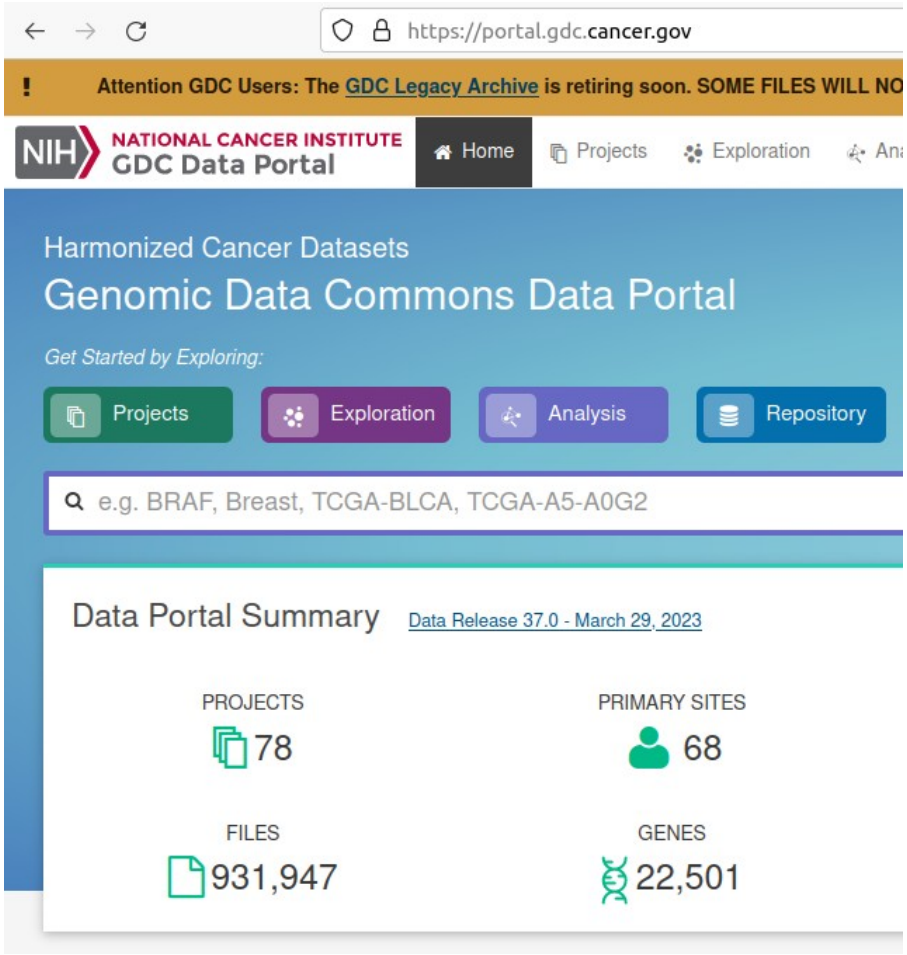
Group your runs/analysis into datasets and link them to your new or existing DAC and policy using **Submitter Portal** or an **XML based programmatic** submission. Data request are done at a dataset level, thus files within a datasets must share release conditions.

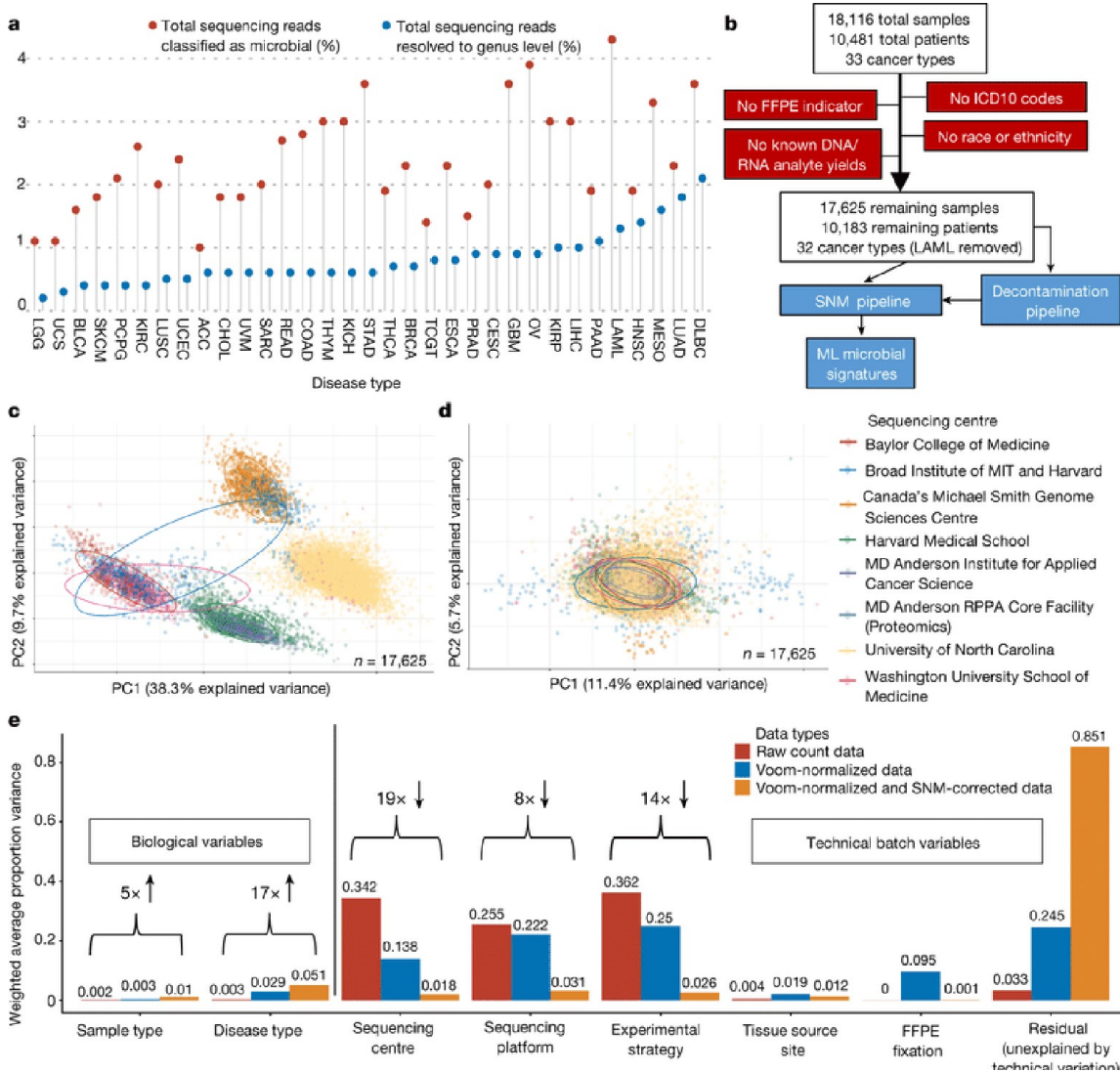
Release and admin

Instruct our Helpdesk to release your study. All registered studies are automatically placed on hold until the named submission or DAC contact instructs our Helpdesk for the study to be released.

6. After data analysis: Publication and FAIR sharing of data.

You source data:





Poore GD et al.: Microbiome analyses of blood and tissues suggest cancer diagnostic approach. Nature 2020.

Store data forever?

Thank you!

Sophia Derdak

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