Medical Genomics Practical #1: Building reproducible workflows

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Rare Cancers Genomics Team

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International Agency for Research on Cancer

World Health Organization

RARE CANCERS GENOMICS

Plan

Part I. Practicals: generating a multi-omic Tumor Map of rare lung tumors

- **Concepts**: Lung Neuroendocrine Neoplasms
- **Data**

Part II. Introduction to domain-specific languages for bioinformatics

- **Concepts**: Reproducibility, scalability, portability
- **Exercises:** run a simple script

Part III. Create your first nextflow script

- **Concepts**: channels and processes
- **Exercises:** create a script printing "hello world"

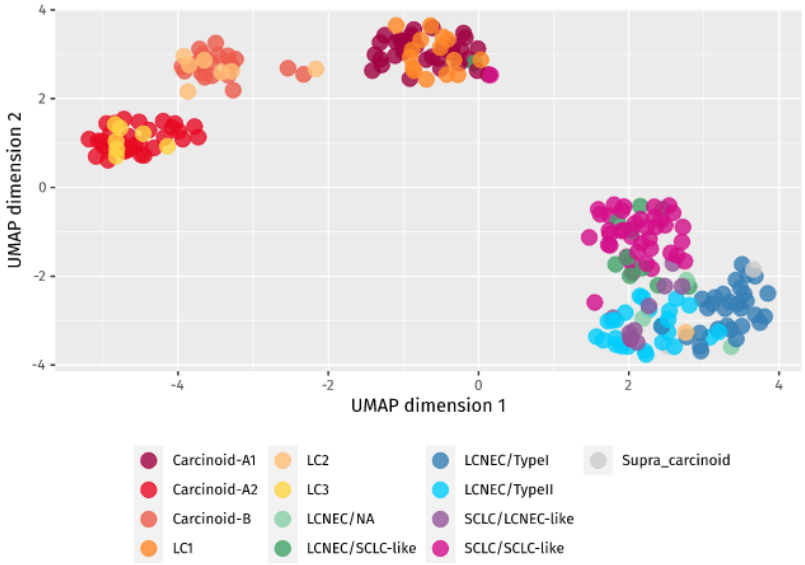
Part IV. Code a simple RNA-seq processing workflow

- **Concepts**: publish output, chaining processes, debugging
- **Exercises:** code a pipeline with multiple steps

Part I. Medical Genomics practicals | *General goal*

Generating a multi-omic Tumor Map of rare lung tumors

- **Lung Neuroendocrine Neoplasms** (LNENs) are rare solid cancers originating from pulmonary neuroendocrine cells
- They are classified (WHO) into grade 1 (typical carcinoids), grade 2 (atypical carcinoids), and grade 3 (carcinoma)
- **Multi-omic datasets** (exomes, RNA-seq, and methylation arrays) were recently generated
- **Clinically relevant molecular groups** with different prognosis (from 88% to 30% 10-year survival) and potential therapeutic targets



Tumor Map (UMAP) of lung neuroendocrine neoplasms. *Source:* https://nextjournal.com/rarecancersgenomics/a-molecular-map-of-lung-neuroendocrine-neoplasms/ *(Gabriel AAG*, Mathian E*, et al. GigaScience 2020). *Equally contributing.*

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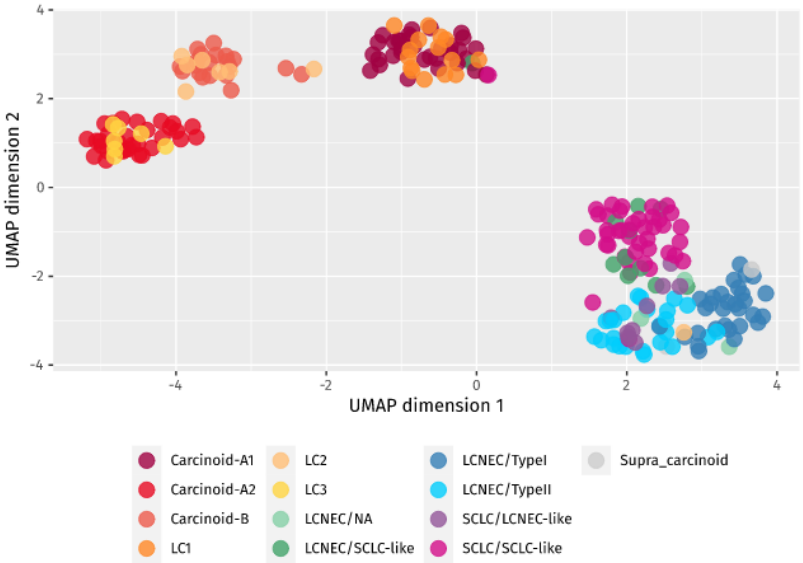
Part I. Medical Genomics practicals | *General goal*

Generating a multi-omic Tumor Map of rare lung tumors

- **Weeks 1-2: build a workflow to pre-process the RNA-seq data and obtain a gene expression matrix**
- **Week 3-4: generate a "tumor map" from the gene expression and gene methylation matrices, analyze the results**



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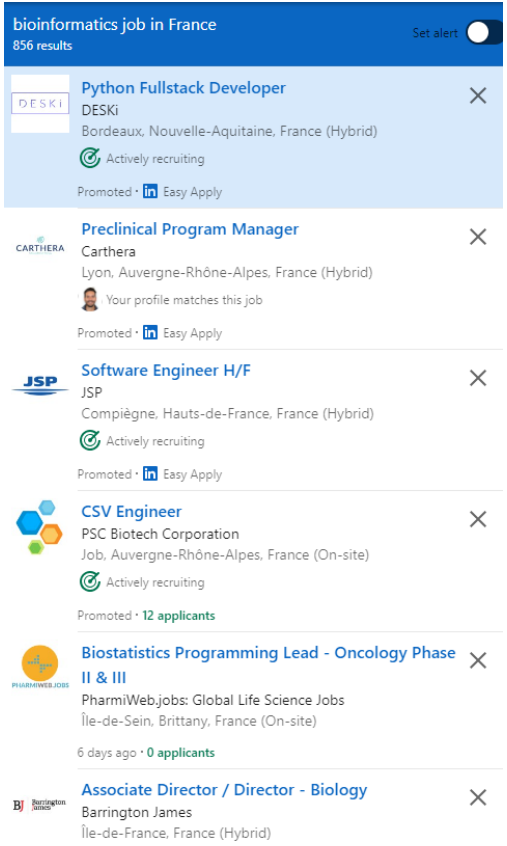
Part II. Introduction to DSL for bioinformatics | *Careers*

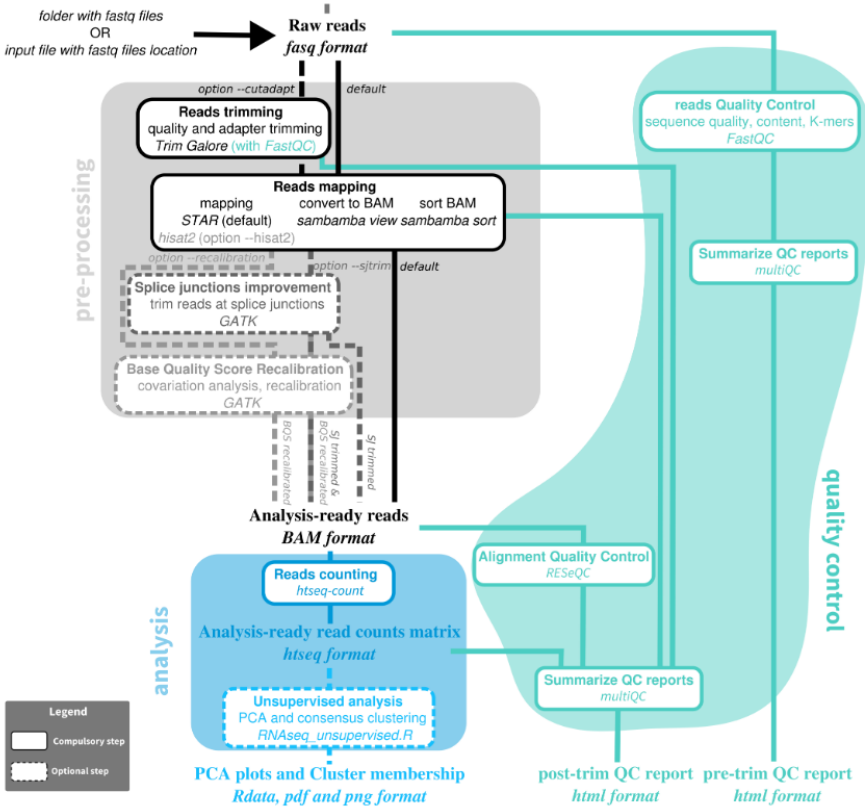
- Computational biology/bioinformatics careers range from **software engineering** to **data management/processing** and **data science**



Part II. Introduction to DSL for bioinformatics | *Example*

RNA-sequencing processing (IARCbioinfo/RNAseq-nf)

- Multiple tasks have to be performed in succession (e.g., trimming, mapping, quantification) or in parallel (on each sample)
- Each task uses its own software and libraries, and can have incompatible requirements (e.g., python versions)



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Part II. Introduction to DSL for bioinformatics | *Concepts*

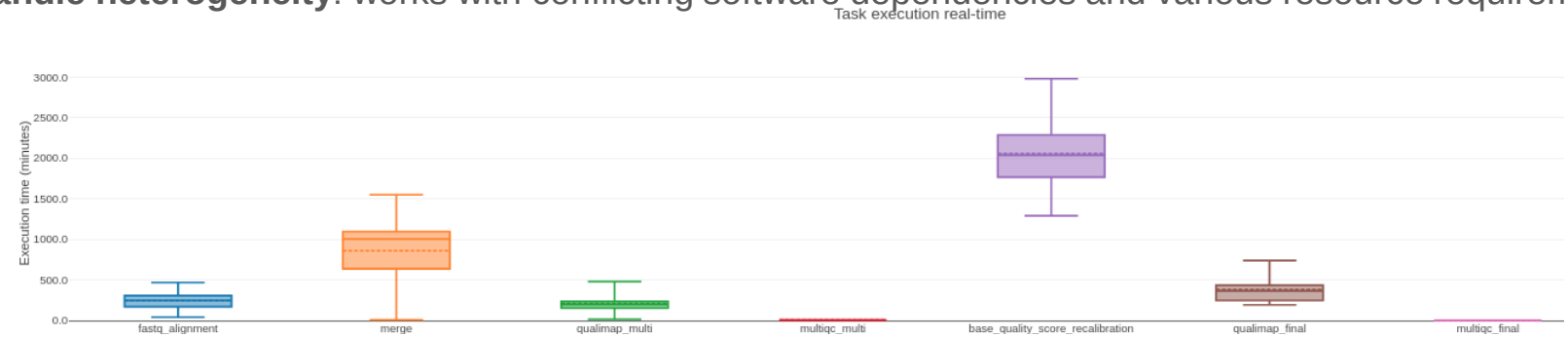
Workflow requirements for large-scale medical genomic projects

Reproducible: clinical and research applications need to be entirely reproducible

Scalable: easily run on large High-Performance Computing facilities

Portable: can run on various infrastructures (different OS, cloud)

Handle heterogeneity: works with conflicting software dependencies and various resource requirements



Example of duration of pre-processing. Tumor/normal pairs whole-genome sequencing (30X and 90X, respectively) for 10 patients, processed using workflow IARCbioinfo/alignment-nf (bwa+post-alignment+GATK BQSR+ pre- and post-alignment QC). **Total of 28,862.4 CPU hours (3 years and 3.5 months), actually processed in 3 days and 18 hrs.**

Part II. Introduction to DSL for bioinformatics | *Concepts*

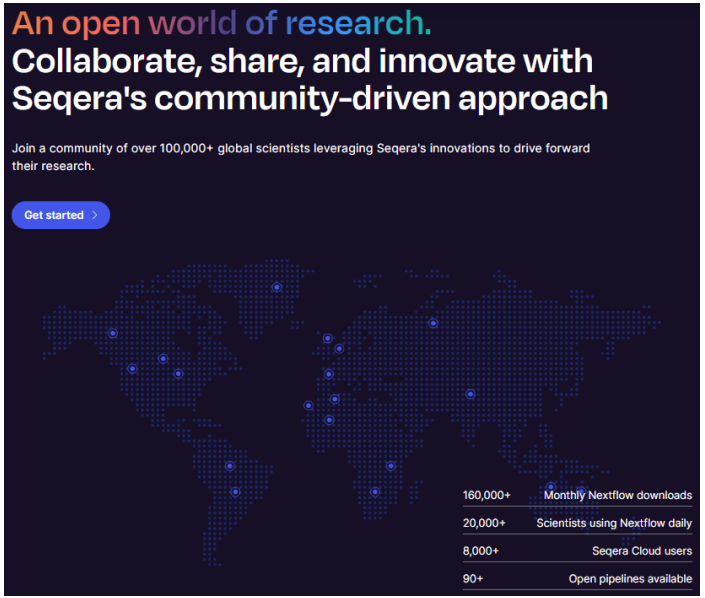
Solutions: domain-specific languages for bioinformatics

High-level programming languages designed to build workflows

- **Nextflow**: based on groovy (java); made at Centre for Genomic Regulation (Barcelona) / Seqera labs
- Snakemake: based on python; made at Institute of Human Genetics (Essen)
- WDL: own language; made at BROAD institute (Boston)

Part II. Introduction to DSL for bioinformatics | *Concepts*

Seqera labs (https://seqera.io/) Nextflow



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Part II. Introduction to DSL for bioinformatics | *Concepts*

Nextflow design

1. Write code in any language

```
trim_galore --paired --fastqc --gzip \
  --basename sample1 -j 2 pair_1.fq pair_2.fq
```

2. Define succession of processes using dataflow programming

`Trimming | Mapping | Quantification`

3. Define software dependencies

`Conda, Docker, Singularity`

4. Define configuration

`Executor (local, HPC scheduler), error management, etc`

5. Run

Part II. Introduction to DSL for bioinformatics | *Concepts*

Nextflow language features

Dataflow language with implicit parallelization and scheduling of tasks:

- the **same set of tasks is applied to all input files**
- tasks are **automatically executed in the proper order** given their inputs and outputs
- **automatically handles submission** of jobs to HPC scheduler

Part II. Introduction to DSL for bioinformatics | *Concepts*

Running a workflow

```
nextflow run IARCbioinfo/pipeline-nf -profile conda --input_folder input/
```

Workflow name: either the *path to a nextflow script*, a *path to a directory* containing a nextflow config file pointing to a nextflow script, or a *github repository* (here, https://github.com/IARCbioinfo/pipeline-nf)

Nextflow options ("-"): e.g., profiles defining location of conda recipes listing software dependencies

Workflow options ("--"): e.g., input files location

Part II. Introduction to DSL for bioinformatics | *Practice*

Practical 1. Questions 1-3

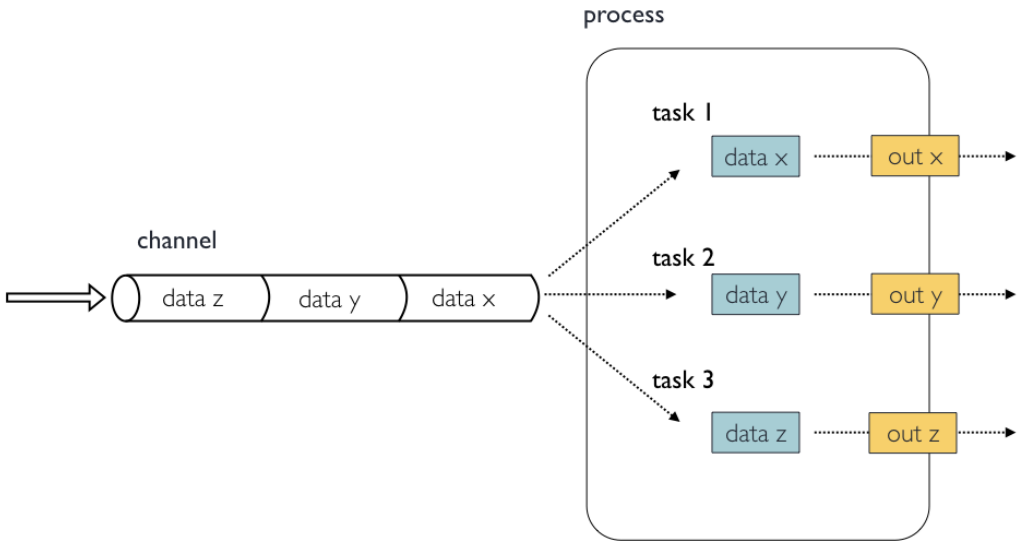
https://github.com/IARCbioinfo/medical_genomics_course/wiki/Practical-1

Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **Channel:** array storing process inputs or outputs (e.g., paths to data)

- **Processes:** instructions to execute a task on an input channel element
  - Note: Nextflow creates a work directory for each task, creates symbolic links to paths from Channel and executes the script defined in the process



Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **Channel:** array storing process inputs or outputs (e.g., paths to data)

Example: test.nf containing

```
TOTO = Channel.from("toto") //creates channel
              .view()       //prints channel
```

```
> nextflow run test.nf
N E X T F L O W  ~  version 20.10.0

Launching `test.nf` [loving_roentgen] -
revision: 9634ca93db

toto
```

Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **Processes:** instructions to execute a task on an input channel element
  - name
  - input channel(s) name(s) and type(s)
  - output channel(s) name(s) and type(s)
  - script (code to be executed)

```
process test{
    input:
    path bam

    output:
    path "${bam}.name"

    shell:
    '''
    echo "!{bam}" > !{bam}.name
    '''
}
```

Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **Processes:** instructions to execute a task on an input channel element
  - input channel(s) type(s) and name(s)
  - output channel(s) type(s) and name(s)
  - types: *path (absolute path of files), val (for variables, e.g., strings & numbers), tuple (multiple values of different types), stdout (everything printed by the script)*
  - *supports wildcards*
  - *"$" to use nextflow variables*
  - *output paths are relative to the work folder*

```
process test{
        input:
        path bam

        output:
        path "${bam}.name"

        shell:
        '''
        echo "!{bam}" > !{bam}.name
        '''
}
```

Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **Processes:** instructions to execute a task on an input channel element
  - script (code to be executed)
  - “!” is used for nextflow variables (e.g., input variables) and “$” for bash variables

```
process test{
        input:
        path bam

        output:
        path "${bam}.name"

        shell:
        '''
        echo "!{bam}" > !{bam}.name
        '''
}
```

Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **workflows:** order of processes to execute (similar to "main" in C language)

```
bam_ch = channel.fromFile("file1.bam")

process test{
...
}

workflow {
 test(bam_ch)
}
```

Part III. Coding a simple workflow | *Basics of Nextflow*

Coding a workflow

- **Nextflow scripting language:**
  - groovy syntax + nextflow-specific objects and operators
  - see more at https://www.nextflow.io/docs/latest/script.html#script-page

Part III. Coding a simple workflow | *Write a simple script*

Practical 1. Question 4

https://github.com/IARCbioinfo/medical_genomics_course/wiki/Practical-1

Part III. Coding a simple workflow | *Write a simple script*

Practical 1. Question 4

- **Correction**

```
params.greeting = "Hello World!"
greetch = channel.from(params.greeting)

process sayhello(){
        input:
                val x
        output:
                stdout
        shell:
        '''
        echo !{x}
        '''
}
workflow {
   sayhello(greetch).view()
}
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow

- **Channel:** array storing process inputs or outputs (e.g., paths to data)
- **Channel factory:**
  - create a channel from a file path (note: can contain wildcards): `Channel.fromPath('data_test/BAM/*.bam')`

```
> N E X T F L O W  ~  version 20.10.0
Launching `test.nf` [deadly_yalow] - revision: e4af11bd16
/home/nalcala/data_test/BAM/NA06984_N.bam
/home/nalcala/data_test/BAM/NA06984_T.bam
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow

- **Channel:** array storing process inputs or outputs (e.g., paths to data)
- **Channel factory:**
  - create a channel from file pairs:
  `Channel.fromFilePairs('data_test/BAM/*.{bam,bam.bai}')`
  - see complete list of channel constructors at https://www.nextflow.io/docs/latest/channel.html#channel-factory

```
> N E X T F L O W  ~  version 20.10.0
Launching `test.nf` [mighty_rubens] - revision: 8e42d3ebe8
[NA06984_N, [/home/nalcala/data_test/BAM/NA06984_N.bam, /home/nalcala/data_test/BAM/NA06984_N.bam.bai]]
[NA06984_T, [/home/nalcala/data_test/BAM/NA06984_T.bam, /home/nalcala/data_test/BAM/NA06984_T.bam.bai]]
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow

- **Channel:** array storing process inputs or outputs (e.g., paths to data)
- **Channel factory:**
  - see complete list of channel constructors at https://www.nextflow.io/docs/latest/channel.html#channel-factory
- **Notes:**
  - *queue* Channels are consumed each time they are used
  - *singleton* Channels are reusable; created with `file()` and some other operators

```
ref = file("reference.fasta")
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow

- **Processes:**
  - multiple inputs can either be different channels (usually, one queue and all others are singletons) OR a tuple (vector) with multiple matching values

```
process test{
    input:
    path bedfile
    tuple val(name), path(bam)

    ...

}
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow

- **Processes:**
  - multiple inputs can either be different channels (usually, one queue and all others are singletons) OR a tuple (vector) with multiple matching values
  - additionally, input and output elements can themselves be vectors of similar types; elements can be accessed using [index]

```
process test{
        input:
        path bedfile
        path bambai

        ...
        shell:

        '''

        echo "!{bambai[0]} !{bambai[1]}"

        '''

}
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow 2

- **Processes:**
  - (optional) path to output directory
  By default, makes a *symbolic link* to the path of the actual output; can change behavior by specifying a “mode” (e.g., copy, move)
  `publishDir "output", mode: ‘move’`
  Note: once moved, the data is inaccessible so needs to be done at the very end of the script

```
process test{
        input:
        path bam

        output:
        path "${bam}.name"

        publishDir "output"
        shell:
        '''
        echo "!{bam}" > !{bam}.name
        '''
}
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow 2

- **Processes:**
  - (optional) path to output directory
  By default, makes a *symbolic link* to the path of the actual output; can change behavior by specifying a "mode" (e.g., copy, move)
  `publishDir "output", mode: 'move'`
  *Note: **other directives** include the memory usage or number of cpus to use (see list at* https://www.nextflow.io/docs/latest/process.html#directives*)*

```
process test{
        input:
        path bam

        output:
        path "${bam}.name"

        publishDir "output"
        shell:
        '''
        echo "!{bam}" > !{bam}.name
        '''
}
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow 3

- **workflows:** order of processes to execute (similar to "main" in C language), can use "piping" (|) syntax to chain processes

```
Nextflow.enable.dsl=2

bam_ch = channel.fromFile("file1.bam")

process preprocess{
...
}

process quantify{
...
}

workflow {
 preprocess(bam_ch) | quantify
}
```

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Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Coding a workflow 3

- **workflows:** order of processes to execute (similar to "main" in C language), can use "piping" (|) syntax to chain processes OR .out to get the output

```
Nextflow.enable.dsl=2

bam_ch = channel.fromFile("file1.bam")

process preprocess{
...
}

process quantify{
...
}

workflow {
 preprocess(bam_ch)
 quantify(preprocess.out)
}
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Passing parameters to nextflow script

- **Any variable VAR defined as "params.VAR" can be set at execution using flags "--var"**
- Its default value is set at declaration

Example script:

```
params.input_folder = "default_dir/"
Inputs = Channel.fromPath(params.input_folder)
...

> Nextflow run test.nf --input_folder BAM/
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Debugging a nextflow script

- **If error in the nextflow code:** usually an error is returned right away, processes are not executed; to debug: print values and channels (remember to duplicate with operator ".into()" so the printed channel is not consumed)
- **If error in script:** visit work directory created by nextflow and check files
  - *.command.sh*: actual script run
  - *.command.log, out, err*: log, output and error of script

```
name: N E X T F L O W  ~  version 20.10.0
Launching `test.nf` [extravagant_heisenberg] - revision:
d3ac3534eb
executor >  local (2)
[b9/d63363] process > test (2) [100%] 2 of 2, failed: 2 ✗
Error executing process > 'test (1)'
Caused by:
  Missing output file(s) `NA06984_T.bam.name` expected by
process `test (1)`
Command executed:
  echo "!{bam}" > !{bam}.name
Command exit status:
  0
Command output:
  (empty)
Work dir:
/home/nalcala/Medical_Genomics_TP1/work/b1/9dc097b6e17e218
52f1e969075a92d
```

Part IV. Coding an RNA-seq workflow | *Basics of Nextflow*

Resuming an execution

- **The "-resume" flag restarts restarts the execution**
- It checks which tasks were completed (they are cached)
- Processes with **changed parameters** will be re-run
- Processes with **modified code** will be re-run
- *Note: to be able to resume execution, nextflow keeps all intermediate files in its work directory, which needs to be cleaned periodically*

```
> Nextflow run test.nf -resume
```

Part IV. Coding an RNA-seq workflow | *Write a workflow*

Practical 1. Questions 5-7 + bonus questions

https://github.com/IARCbioinfo/medical_genomics_course/wiki/Practical-1

Part V. Coding an RNA-seq workflow | *Basics of Nextflow*

Being a nice user!

- **Computing facilities are shared resources, everyone needs to be mindful**

```
> export NXF_WORK=/temp/nalcala
```

Best practices:

- Work directory should be in the temporary folders (not $HOME), e.g., scratch or temp folders
- Use nextflow environment variables to set the right paths
- Always specify “cpus” and “mem” directives, and adapt resource usage to the system

Part VI. Going further | *Advanced Nextflow*

Interaction with github

- github allows **open code sharing**, **versioning, automated tests**
- **Nextflow can use specific revisions from github, corresponding to branches, tags, or commit IDs**

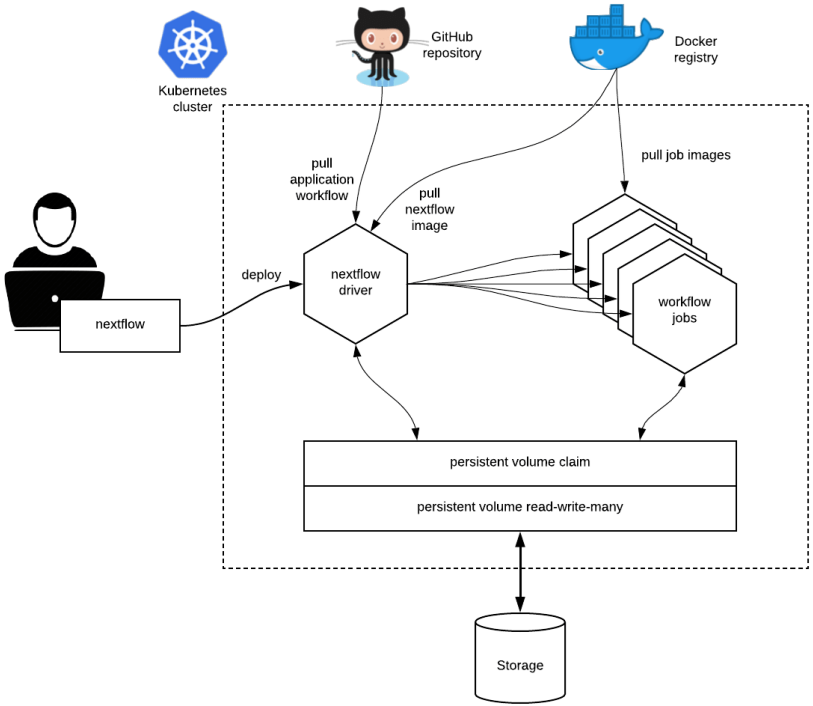
```
> Nextflow run IARCbioinfo/FastQC-nf -r v1.1 --help

> Nextflow run IARCbioinfo/FastQC-nf -r 06365b4dd152d586d2fb9e128c0188a5f84fd257 --help
```

Part VI. Going further | *Advanced Nextflow*

Use on other facilities

- Simply **setting the executor to the HPC scheduler** (e.g., lsf, slurm) enables using the HPC
- Can also be configured to use cloud resources (amazon web services, kubernetes, ...)



Part VI. Going further | *Advanced Nextflow*

- Possibility of using **modules** (external scripts containing some functions)
- Nextflow tower.nf provides an interface to **monitor job execution** and now also **launch jobs** (similar to Galaxy)

```
nextflow.enable.dsl=2

include { foo } from './some/module1'
include { bar } from './some/module2'

workflow {
   channel.from('Hello') | map { it.reverse() } | (foo &
bar) | mix | view
}
```

Part VI. Going further | *Advanced Nextflow*

Configuring nextflow

- **nextflow.config files:** contains options
  - nextflow concatenates config file given using the “-c” flag with the *nextflow.config* from current directory, that from the workflow directory and the global config at $HOME/.nextflow/config
  - useful to define *profiles* with options to run on different infrastructures
  - multiple profiles can be provided using the “-p” flag (e.g., “nextflow run -p conda -p SLURM_IARC”)

```
profiles {
  conda {
          process.conda =
"$baseDir/environment.yml"
  }
  Singularity {
          singularity.enabled = true
          process.container =
'shub://IARCbioinfo/fastqc-nf:v1.1'
  }
  SLURM_IARC {
          process.executor = “slurm”
          queue = “low_p”
          queueSize = 50
  }
}
```

Part VI. Going further | *Advanced Nextflow*

Configuring nextflow

- **nextflow.config files:** contains options for running nextflow.
- **Best practices for reproducibility:** create a conda recipe (yml file) containing all software dependencies

File environment.yml:

```
name: GATK4
channels:
  - bioconda
  - conda-forge
dependencies:
  - gatk4=4.1.5.0
```

Medical Genomics Practical #1: Building reproducible workflows

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Appendix

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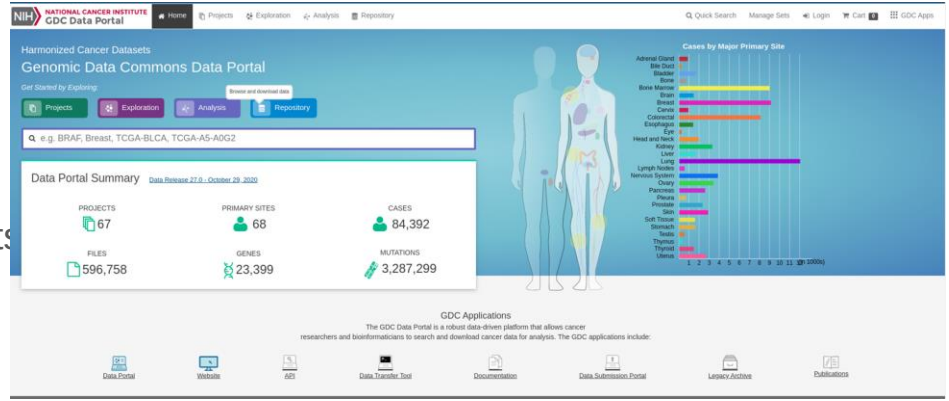
Part I. Transcriptomics | *Ressources*

The Cancer Genome Atlas (TCGA) project

Database of cancer multi-omic data for

- Tumors from 33 primary sites
- RNA-seq data under controlled access (requires research institute affiliation)
- Processed gene expression data (read counts and FPKM) open-access



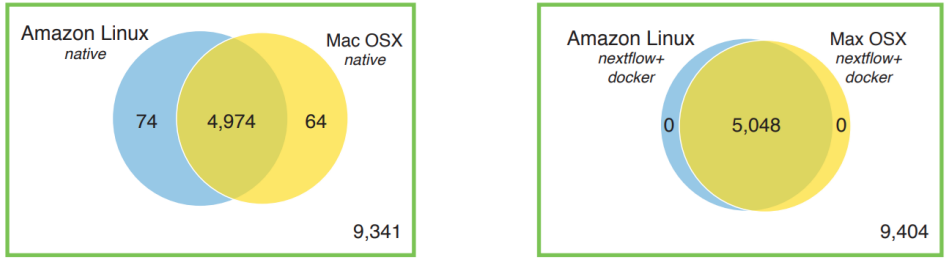
Web interface of the genomic data portal hosting the TCGA data. *Source:* https://portal.gdc.cancer.gov/.

Part I. Transcriptomics | *Techniques*

Bulk sequencing: processing

Notes:

- *RNA-seq analyses are known to suffer from a lack of robustness, so* ***reproducibility and open science practices are of the uttermost importance****!*
- *Even subtle numerical instability issues can impact the results (****Figure****)*
- *See* ***Practical 1*** *for a solution to provide entirely reproducible workflows*



Reproducibility of RNA-seq differential expression analysis of Human Lung Fibroblasts. **Left.** Venn diagram of differentially expressed genes using the exact same versions of all softwares (kallisto and sleuth) but two different OS (blue: a Linux system from AWS, yellow: Mac OS). **Right.** Same as on the left, but running the entire data processing and analysis workflow inside a docker container using the Nextflow language. *Source: Di Tommaso et al. Nature Biotechnology 2017.*

Part I. Transcriptomics | *Ressources*

The Cancer Genome Atlas (TCGA) project

Database of cancer multi-omic data for

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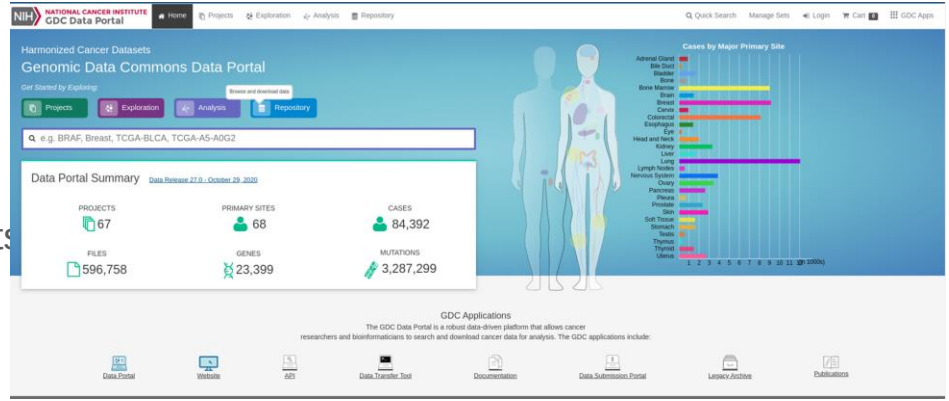


Web interface of the genomic data portal hosting the TCGA data. *Source:* https://portal.gdc.cancer.gov/.

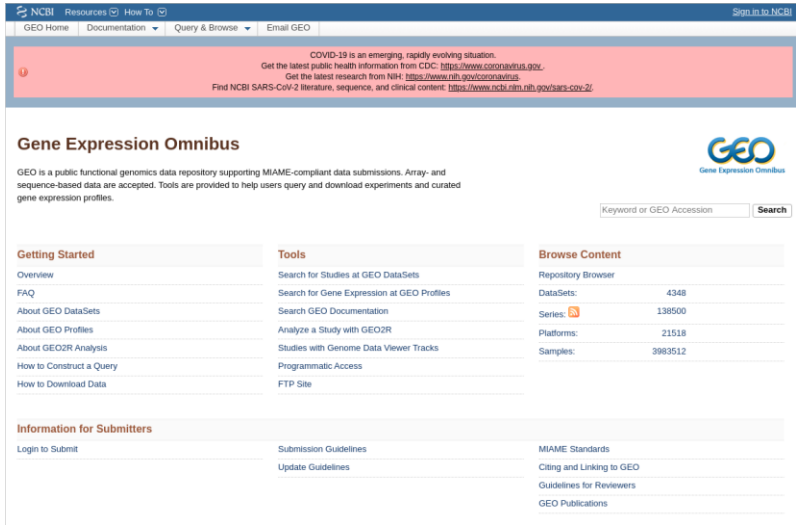
Part I. Transcriptomics | *Ressources*

The Gene Expression Omnibus (GEO) repository

Database of expression data (arrays and RNA-seq)

- Includes human data
- All data is open-access

Will be used for the practicals.



Web interface of the gene expression omnibus repository. *Source:* https://www.ncbi.nlm.nih.gov/geo/.