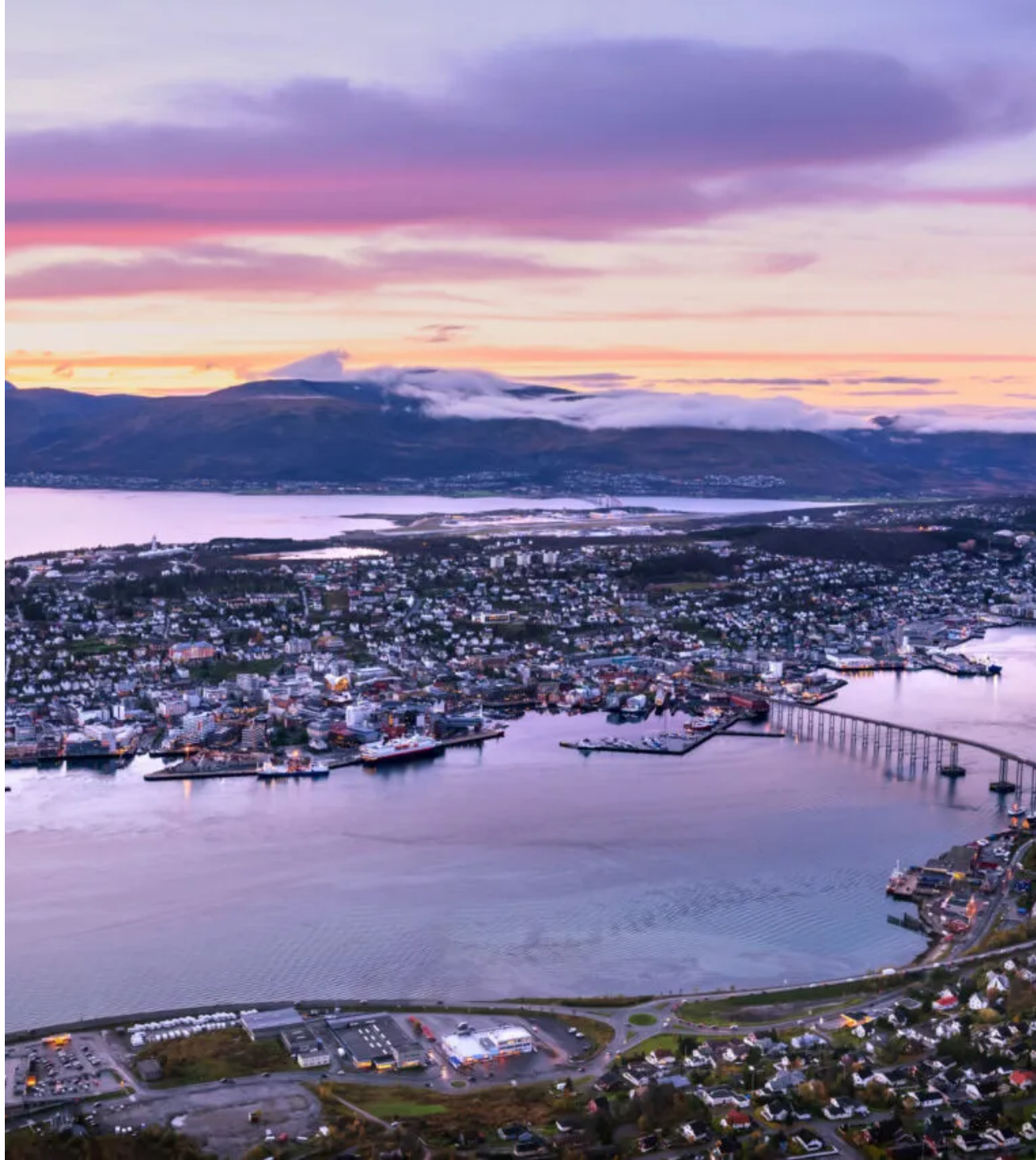
Bioinformatics session

3rd Annual workshop on bioinformatics and variant interpretation in InPreD

https://inpred.github.io/25-06_bioinfo_ws/bioinfo_ws



1. Unit testing

What is unit testing?

- test smallest piece of code that can be logically isolated in software application (function, subroutine, method)
- the smaller the better - more granular view of what is going on; also faster
- should not cross systems (database, filesystem, network) -> integration and functional tests

Example

```
# calculator.py
def add(x, y):
    """add numbers"""
    return x + y
```

```
# test_calculator.py
import calculator

def test_add():
    assert calculator.add(1, 2) == 3
```

Why do we need unit testing?

- early defect detection
- code quality improvement
- facilitates refactoring
- faster development cycles
- better documentation
- enables more frequent releases

How to design a unit test?

- identify the unit (function, method)
- what is its functionality?
- what is the input (correct and incorrect)?
- how to handle incorrect input? (edge cases, invalid data)
- what does it return?
- positive and negative results should be tested

Set up unit testing for your functions

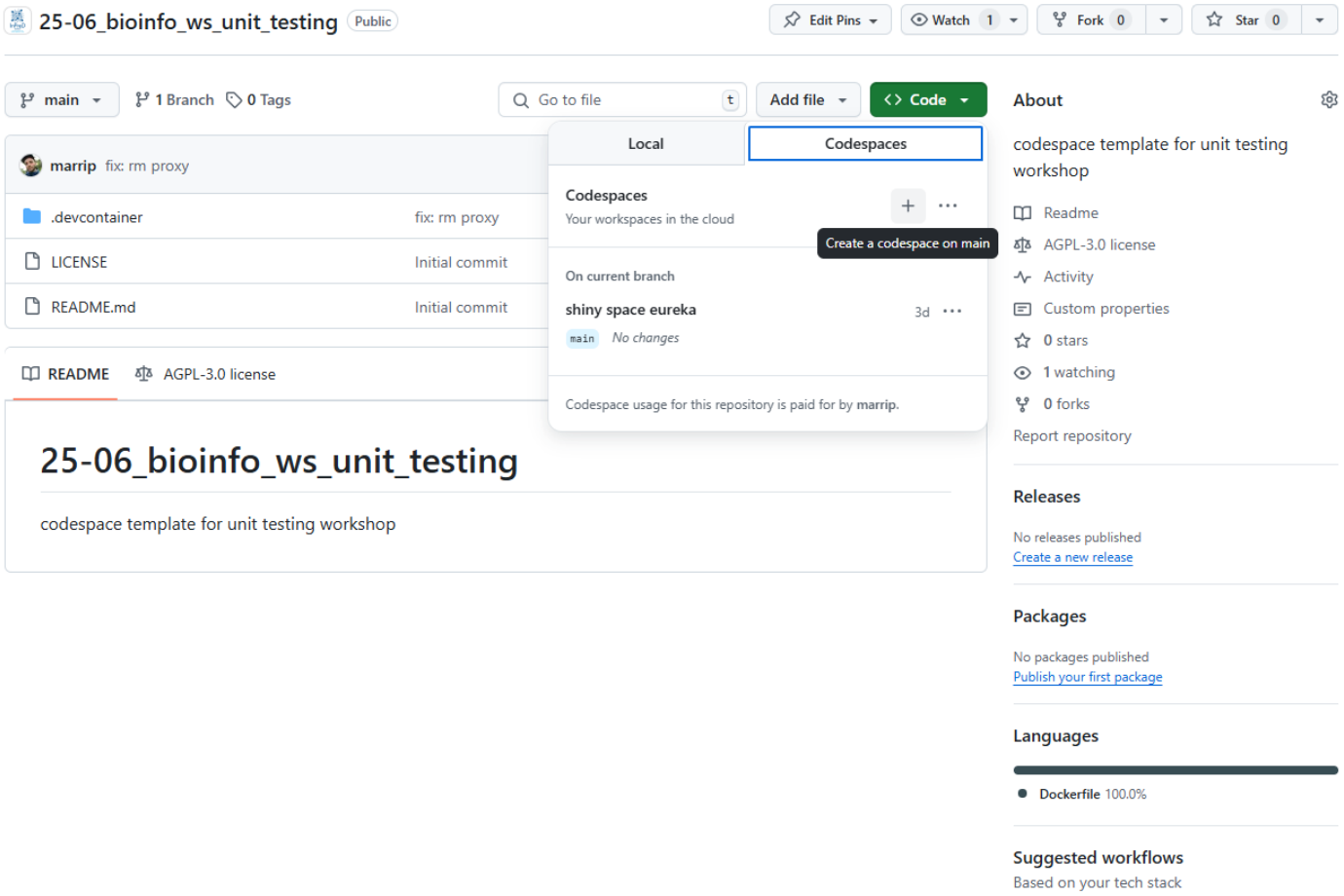
- install pytest

  ```
  $ pip install pytest
  ```

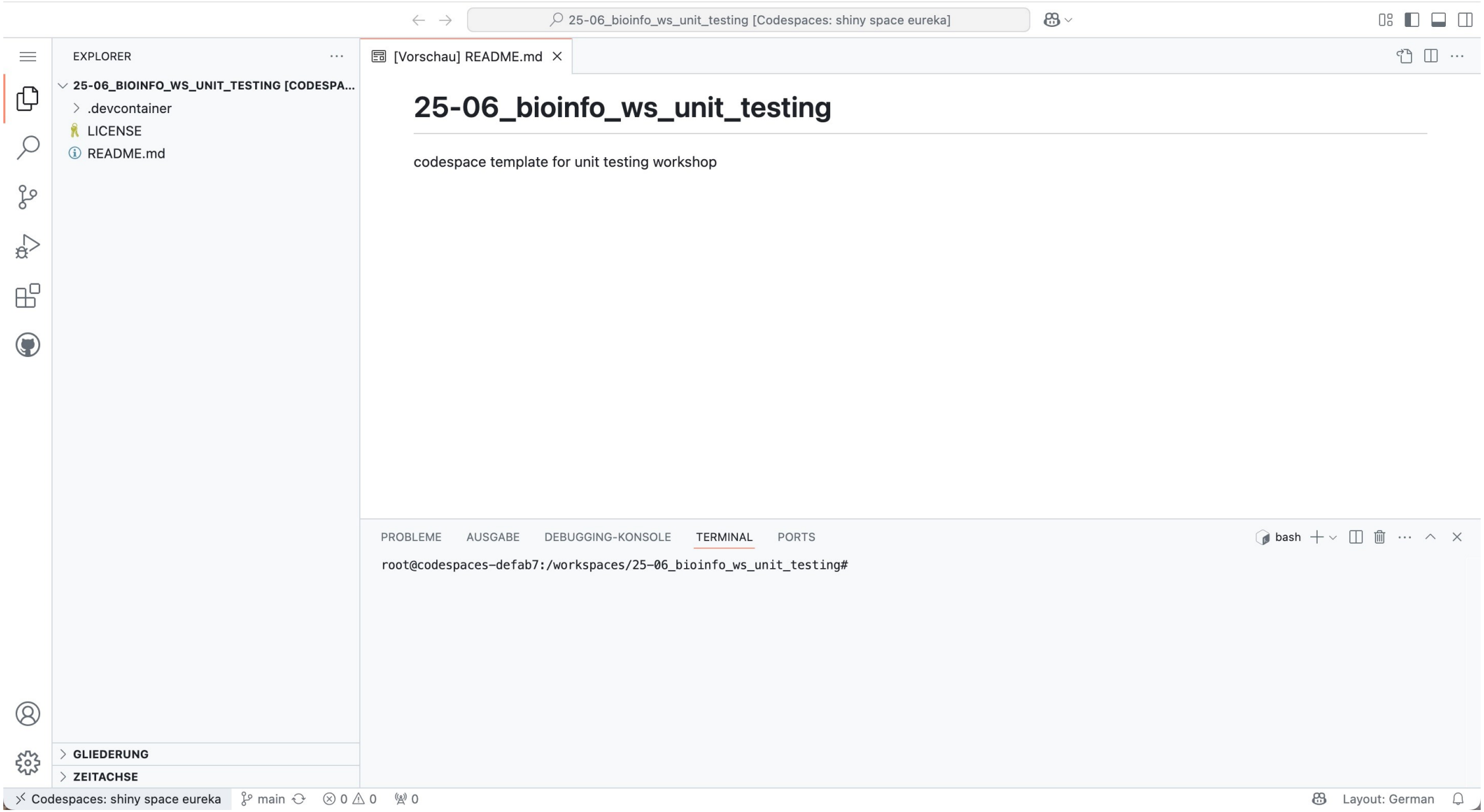
- add your function to a module at `my_module/my_module.py`
- add your unit test at `my_module/tests/my_module_test.py`
- in the test file import your module `from my_module.my_module import my_function`

First exercise

- go to https://github.com/InPreD/25-06_bioinfo_ws_unit_testing



First exercise



First exercise

- pytest was already installed in the codespace
- the suggested layout was already applied
- create a branch for your work:

```
$ git checkout -b unit-tests-<your name>
```

- start with the first exercise in `first/tests/first_test.py`
- whenever you are done, commit your changes (use commit message conventions):

```
$ git add first/tests/first_test.py
$ git commit -m "test: <your commit message>"
```

- and we push them to GitHub:

```
$ git push --set-upstream origin unit-tests-<your name>
```

Handle exceptions in unit tests

- functions can raise exceptions and we would like to test for those
- import `pytest` to have access to `raises()`
- add `with`-block to handle the exception:

```
import calculator
import pytest

def test_add_exception():
    with pytest.raises(TypeError):
        assert add("one", "two") == None
```

Second exercise

- continue with the second exercise in `second/tests/second_test.py`
- whenever you are done, commit your changes (use commit message conventions):

```
$ git add second/tests/second_test.py
$ git commit -m "test: <your commit message>"
```

- and we push them to GitHub:

```
$ git push
```

Make unit tests table-driven by using parametrize

- having more than one test case results in repeating a lot of code (one function per test case)
- to condense this as much as possible (ideally one unit test per function), we can use the `pytest` decorator `parametrize`
- again, import `pytest` to gain access to the decorator
- add the decorator `@pytest.mark.parametrize` as a header to your function
- define the required variables (input, exception, output)
- add your test cases as a list of tuples (one tuple per case)
- also use `nullcontext` from the module `contextlib` to account for cases without exceptions

```
import calculator
import pytest

from contextlib import nullcontext

@pytest.mark.parametrize(
    "x, y, exception, want",
    [
        (1, 2, nullcontext(), 3),
        ("one", "two", pytest.raises(TypeError), None)
    ]
)
def test_add(x, y, exception, want):
    with exception:
        assert add(x, y) == want
```

Third exercise

- continue with the third exercise in `third/tests/third_test.py`
- whenever you are done, commit your changes (use commit message conventions):

```
$ git add third/tests/third_test.py
$ git commit -m "test: <your commit message>"
```

- and we push them to GitHub:

```
$ git push
```

Use GitHub action to automatically run tests on push

- add `.github/workflows/main.yml`:

```yaml
name: Python test
on: push

jobs:
  test:
    name: Run unit tests
    runs-on: ubuntu-latest
    steps:
      -
        name: Check out the repo
        uses: actions/checkout@v4
      -
        name: Set up Python 3.12.8
        uses: actions/setup-python@v4
        with:
          python-version: 3.12.8
      -
        name: Install dependencies
        run: pip install -r requirements.txt
      -
        name: Unit testing
        uses: pavelzw/pytest-action@v2
        with:
          verbose: true
          emoji: true
          job-summary: true
          custom-arguments: -q
          click-to-expand: true
          report-title: 'Bioinfo workshop unit testing'
```

Use GitHub action to automatically run tests on push

- if you don't want to write all of that, merge the branch containing the file into your branch:

```
$ git merge add-github-action
```

Fourth exercise

- write unit tests for the functions in `fourth/fourth.py`
- whenever you are done, commit your changes (use commit message conventions):

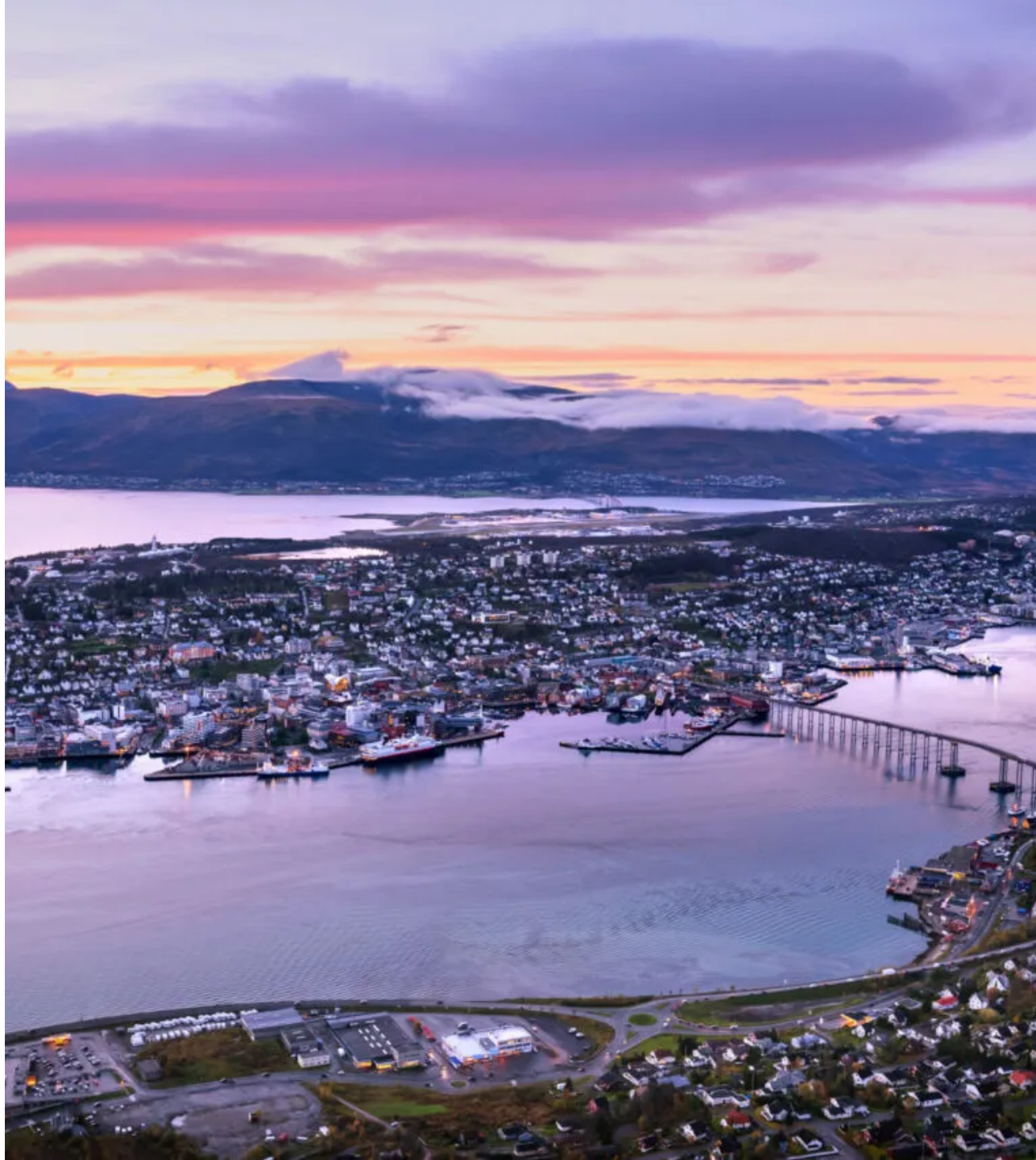
```
$ git add third/tests/third_test.py
$ git commit -m "test: <your commit message>"
```

- and we push them to GitHub:

```
$ git push
```

Thank you for your attention!

Day 1 done!



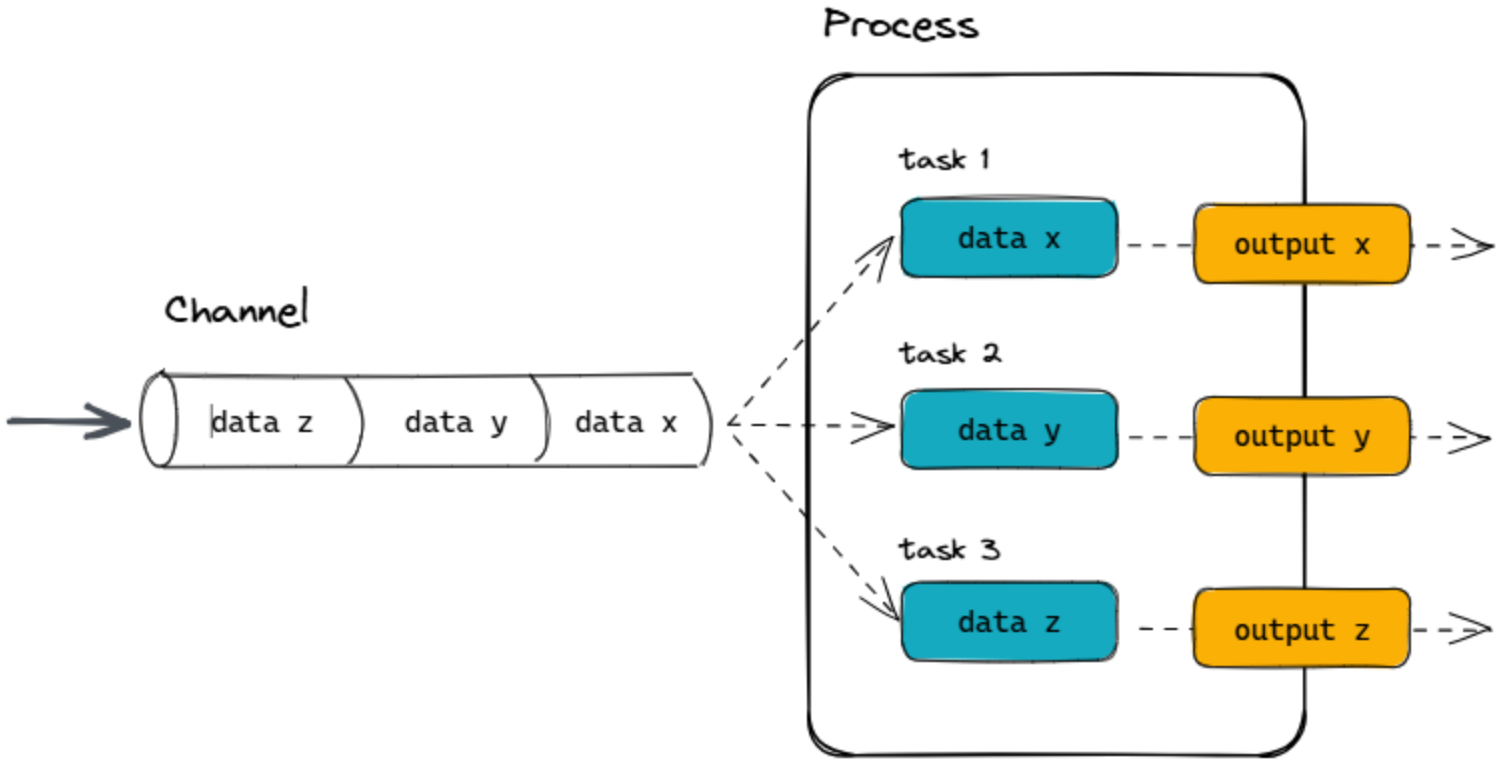
2. Nextflow

What is nextflow?

- workflow orchestration engine, domain-specific language (in contrast to general-purpose language, e.g. python)
- easy to write data-intensive computational workflows
- extension of groovy which is a superset of Java
- core features:
  - portability and reproducibility
  - scalability of parallelization and deployment
  - integration of existing tools, systems, and industry standards

Processes and channels

- different processes joined together - each written in any language that can be executed by Linux platform
- independently and isolated processes - not sharing common (writable) state
- communication via asynchronous first-in, first-out (FIFO) queues, called `channels`

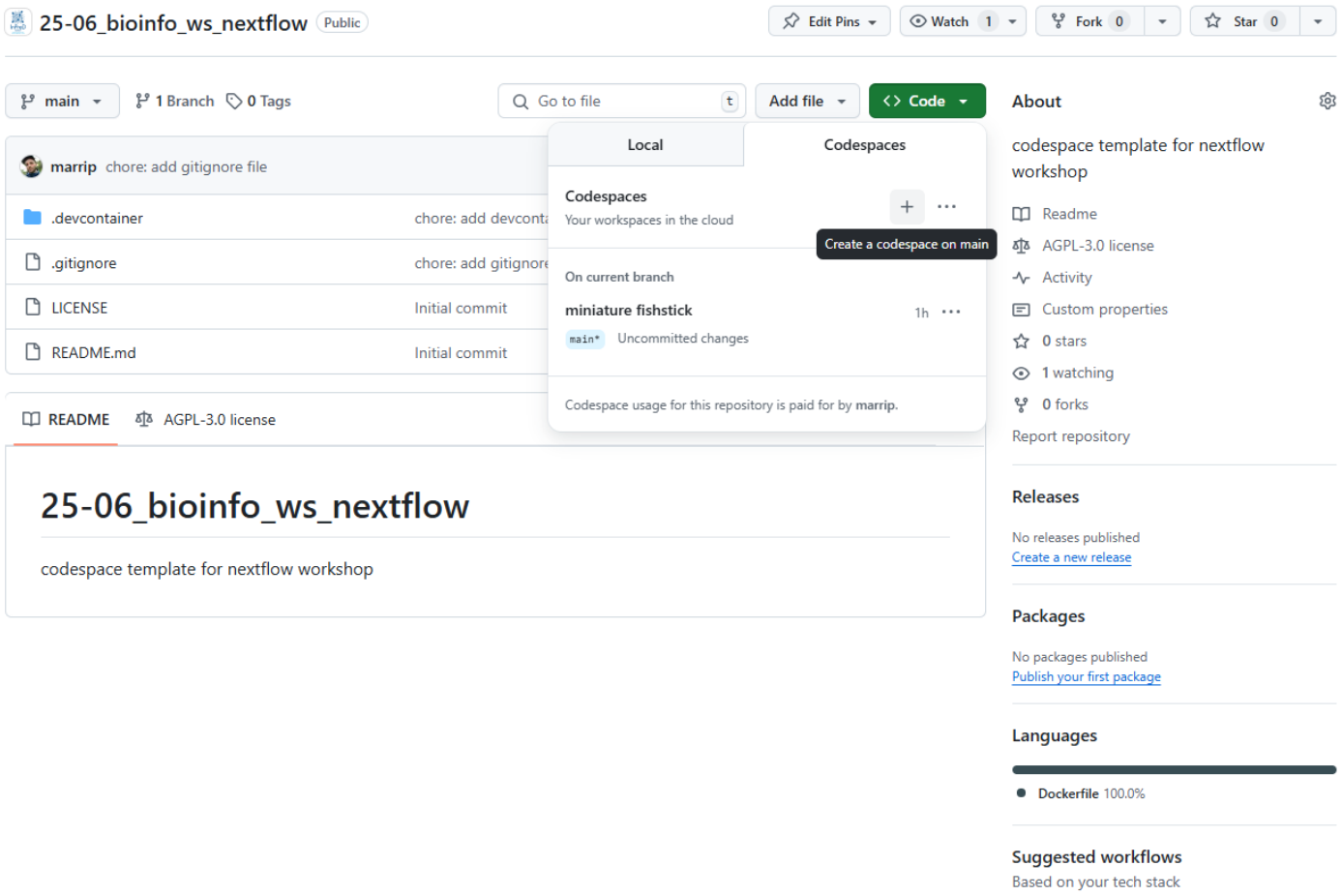


Execution abstraction

- process = *what* is executed <-> executor = *how* it is executed
- provides abstraction between workflow's functional logic and underlying execution system/runtime
- workflow runs seamlessly on local computer, HPC cluster or cloud

Let's get started

- go to https://github.com/InPreD/25-06_bioinfo_ws_nextflow



- create a branch for your work

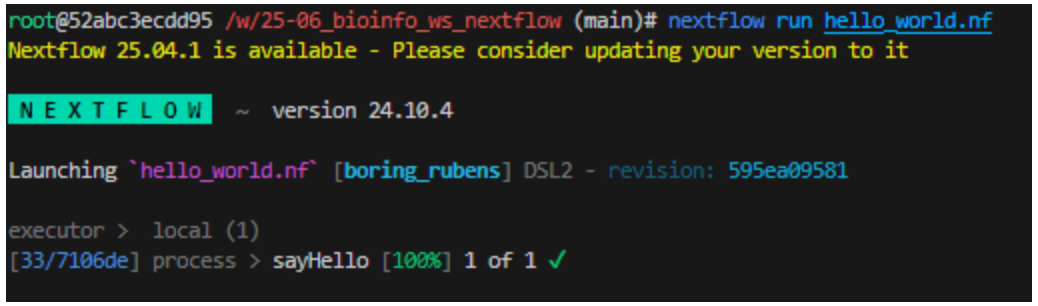
```
$ git checkout -b nextflow-<your name>
```

- create a new file `hello_world.nf`
- write a workflow which outputs a file containing "Hello World!"

```
#!/usr/bin/env nextflow

/*
 * Use echo to print 'Hello World!' to a file
 */
process sayHello {

    output:
        path 'output.txt'

    script:
    """
    echo 'Hello World!' > output.txt
    """
}

workflow {

    // emit a greeting
    sayHello()
}
```

- try to run it

```
$ nextflow run hello_world.nf
```



- check if you can find `work/33/7106de/output.txt`
- explore the other files that are in `work/33/7106de/`

- add a directory to which results should be published

```
process sayHello {

    publishDir 'results', mode: 'copy'

    output:
        path 'output.txt'
    ...
}
```

- add a greeting variable

```
process sayHello {

    publishDir 'results', mode: 'copy'

    input:
        val greeting

    output:
        path 'output.txt'

    script:
    """
    echo '$greeting' > output.txt
    """
}

workflow {

    // emit a greeting
    sayHello(params.greeting)
}
```

```
$ nextflow run hello_world.nf --greeting 'Heisann!'
```

- move the `params.greeting` into the `hello_world.nf`:

```
params.greeting = 'Heisann!'

process sayHello {

    publishDir 'results', mode: 'copy'
    ...
}
```

```
$ nextflow run hello_world.nf
```

```
$ nextflow run hello_world.nf --greeting 'Hejsan!'
```

- transform greetings into a channel

```
process sayHello {

    input:
        val greeting

    output:
        stdout

    script:
    """
    echo '$greeting'
    """
}

workflow {

    greeting_ch = Channel.of('Alo', 'Salut', 'Sunt eu').view()
    // emit a greeting
    sayHello(greeting_ch) | view
}
```

```
$ nextflow run hello_world.nf -ansi-log false # run it several times
```

Channels

- processes communicate through channels
- two major properties:
  - Sending a message is an asynchronous (i.e. non-blocking) operation
  - Receiving a message is a synchronous (i.e. blocking) operation
- please refer to the nextflow docs about channels

- create a `.csv` file containing our greetings which will serve as input

```
Alo
Salut
Sunt eu
```

- create channel from the file using the channel factory `fromPath()`

```
workflow {

    greeting_ch = Channel.fromPath(params.greeting).view()
    // emit a greeting
    sayHello(greeting_ch) | view
}
```

```
$ nextflow run hello_world.nf -ansi-log false --greeting greetings.csv
```

- try to manipulate a channel by using the operators `splitCsv()` and `map()`

```
workflow {

    greeting_ch = Channel.fromPath(params.greeting)
                         .view( it -> "Before splitCsv: $it" )
                         .splitCsv()
                         .view( it -> "After splitCsv: $it" )
                         .map( item -> item[0] )
                         .view( it -> "After map: $it" )
    // emit a greeting
    sayHello(greeting_ch) | view
}
```

```
$ nextflow run hello_world.nf -ansi-log false --greeting greetings.csv
```

- please refer to the nextflow docs about channel operators

- add a second process

```
process convertToUpper {

    input:
        val lower

    output:
        stdout

    script:
    """
    echo '$lower' | tr '[a-z]' '[A-Z]'
    """
}
```

- include the process in the workflow and link it to the first one

```
workflow {

    greeting_ch = Channel.fromPath(params.greeting)
                         .view( it -> "Before splitCsv: $it" )
                         .splitCsv()
                         .view( it -> "After splitCsv: $it" )
                         .map( item -> item[0] )
                         .view( it -> "After map: $it" )
    // emit a greeting
    sayHello(greeting_ch) | view
    convertToUpper(sayHello.out) | view
}
```

```
$ nextflow run hello_world.nf -ansi-log false --greeting greetings.csv
```

- run first process inside a container

```
process sayHello {

    container 'ubuntu:24.04'
    input:
        val greeting

    output:
        stdout

    script:
    """
    echo '$greeting'
    """
}
```

- add `nextflow.config` containing

```
docker.enabled = true
```

- please refer to the nextflow docs on configuration

- check your docker images

```
$ docker images
```

- now run

```
$ nextflow run hello_world.nf -ansi-log false --greeting greetings.csv
```

- check your docker images again

```
$ docker images
```

- restructure you workflow a bit by moving the processes in a `modules/` subfolder
- each process should get its own file, e.g. `modules/sayHello.nf`

```
#!/usr/bin/env nextflow

process sayHello {

    input:
        val greeting

    output:
        stdout

    script:
    """
    echo '$greeting'
    """
}
```

- include the modules in the header of your workflow file

```
include { sayHello       } from './modules/sayHello.nf'
include { convertToUpper } from './modules/convertToUpper.nf'

workflow {
  ...
}
```

```
$ nextflow run hello_world.nf -ansi-log false --greeting greetings.csv
```

nf-core

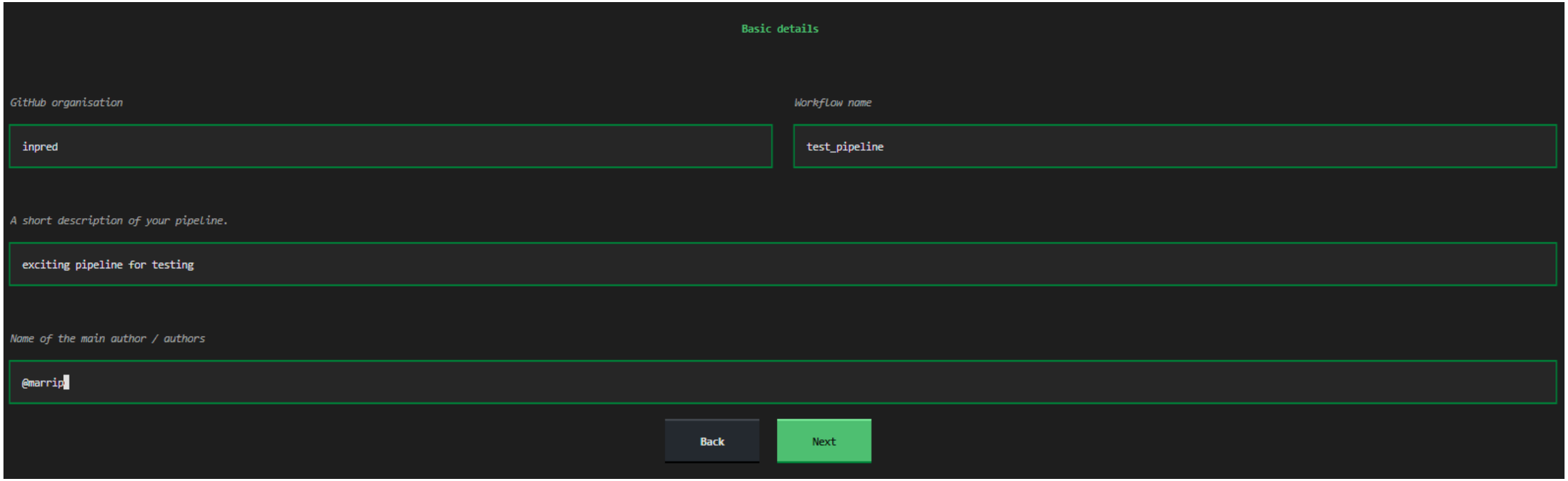
- diverse project spread across many groups (Seqera, SciLifeLab Sweden, Centre for Genomic Regulation etc.)
- community effort to collect a curated set of analysis pipelines built using nextflow
- standardised set of best practices, guidelines, and templates
- modular, scalable, and portable pipelines - researchers can easily adapt and execute them using own data and compute resources
- open development, testing, and peer review -> pipelines are robust, well-documented, and validated against real-world datasets

- 113 nf-core pipelines (October 2024):
  - 68 released
  - 32 under development
  - 13 archived
- please refer to the nf-core website for more information and resources

- create a pipeline template using `nf-core` tooling

```
$ nf-core pipelines create
```

- in the tui we choose `Let's go` > `Custom` **(OBS! Name should not contain `-`)**



- unselect `Toggle all features` and select the following:
  - `Add configuration files`
  - `Use code linters`
  - `Use fastqc`
  - `Use nf-core components`
  - `Use nf-schema`
  - `Add testing profiles`
- continue with `Continue` > `Finish` > `Continue` > `Finish without creating a repo` > `Close`
- take a look at the output

- **stubbing** = quickly prototype the workflow logic without using the real commands; comparable to dry-run
- prepare for a stub run by adding mock fastq file to `assets/`

```
$ touch assets/sample1_R1.fastq.gz assets/sample1_R2.fastq.gz assets/sample2_R1.fastq.gz
```

- update `assets/samplesheet.csv` like so

```
sample,fastq_1,fastq_2
SAMPLE_PAIRED_END,assets/sample1.fastq_R1.gz,assets/sample1_R2.fastq.gz
SAMPLE_SINGLE_END,assets/sample2.fastq_R1.gz
```

- run your first nf-core pipeline

```
$ nextflow run . -stub --input assets/samplesheet.csv --outdir results # -ansi-log false
```

- add the nf-core module `bwa/mem`

```
$ nf-core modules install bwa/mem
```

- add it to `workflows/test_pipeline.nf`

```
include { BWA_MEM                     } from '../modules/nf-core/bwa/mem/main'
include { FASTQC                      } from '../modules/nf-core/fastqc/main'
...
    ch_versions = ch_versions.mix(FASTQC.out.versions.first())

    //
    // MODULE: Run bwa mem
    //
    BWA_MEM (
        ch_samplesheet,
        [[], []],
        [[], []],
        []
    )
```

- in the stub section of `modules/nf-core/bwa/mem/main.nf`, change the version command string to

```
    """
    ...
    cat <<-END_VERSIONS > versions.yml
    "${task.process}":
        bwa: mock
        samtools: mock
    END_VERSION
    """
```

```
$ nextflow run . -stub --input assets/samplesheet.csv --outdir results # -ansi-log false
```

- try to add other modules from `nf-core/modules`, e.g. `trimmomatic` or `cutadapt`, and link them them to `bwa/mem`

Thank you for your attention!

Day 2 done!