Snakemake for reproducible research

Making Snakemake even more reproducible

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What could we improve? (again)

- Using unknown number of inputs/outputs

- Using scripts from other languages

- Being reproducible

What could we improve? (again)

- Using unknown number of inputs/outputs ⟶ Input functions, checkpoints

- Using scripts from other languages ⟶ Directives run and script

- Being reproducible ⟶ conda/mamba, Docker/Apptainer

Working with <u>with</u> an unknown number of inputs/outputs

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  - Input files depend on wildcards
  - Number of input files is hard to determine

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  - Define the function above the rule
  - Use the syntax input: <function_name>
    - No parentheses, no argument

```python
def seq_input(wildcards):
    type = wildcards.type
    if type == 'SE':
        return 'data/file1.fq'
    else:
        return ['data/file1.fq', 'data/file2.fq']

rule merge_files:
    input:
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    output:
        'results/{type}.txt'
    shell:
        'cat {input} > {output}'
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- Input functions = Python functions
  - Single argument: 'wildcards'
  - Return a file or list of files
  - Can also return a dictionary with input names as keys
    - Use input: unpack(<function_name>) to obtain named inputs

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- Functions are evaluated before workflow execution ➔ can't list output files
  - No output functions!

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```

```
snakemake --cores 1 results/not_SE.txt
```

⇩

{type} = "NotSE"

⇩

```
input:
    ['data/file1.fq', 'data/file2.fq']
```

Working <u>after</u> an unknown number of inputs/outputs

- *aka* 'Data-dependent conditional execution' *aka* **checkpoint** (instead of **rule**)

- When:
  - An unknown number of files is generated by a rule
  - Output files are unknown before execution

- Conditional reevaluation of the DAG of jobs based on the outputs content
  - Since DAG is re-evaluated midway ➔ you can't see the whole workflow at the start

- **Very complicated!**

Executing external code in Snakemake

● There are 2 ways to execute external code in Snakemake: run and script

Executing external code in Snakemake: run

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rule get_header:
    input:
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    params:
        lines = 5
    run:
        input_file = open(input[0])
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- Value = path to the script relative to the rule's snakefile

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- Advantages:
  - Great for long code
  - Can use **conda**/**singularity** directive!!!

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    output:
        'results/file_header.txt'
    params:
        lines = 5
    script:
        'first_step.py'
```

first_step.py
```
# Retrieve information from Snakemake
input_file = open(snakemake.input[0])
output_file = open(snakemake.output[0], 'w')
n_lines = snakemake.params.lines

# Process file
for i in range(n_lines):
    output_file.write(input_file.readline())
```

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    params:
        lines = 5
    script:
        'first_step.py'
```

```
                                    first_step.R
library(readr)

# Retrieve information from Snakemake
input_path <- snakemake@input[[1]]
output_path <- snakemake@output[[1]]
n_lines <- snakemake@params$lines[1]

# Process file
data <- read_delim(input_path, '\t', n_max=n_lines)
```

Being reproducible with Snakemake and Conda

- What is conda?

Being reproducible with Snakemake and Conda

- What is conda?

    - **Conda/mamba**: open-source, cross-platform, language-agnostic package manager and environment management system

    - **Channels**: field-specific repositories of software
        - Conda-forge: general computation
        - Bioconda: bioinformatics

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  - ○ **Channels**: field-specific repositories of software
    - ■ Conda-forge: general computation
    - ■ Bioconda: bioinformatics

  - ○ Environments defined in YAML files

```yaml
name: python_env
channels:
    - conda-forge
    - bioconda
dependencies:
    - python >= 3.12
    - pandas == 2.2.3
```
py.yaml

Being reproducible with Snakemake and Conda

- Using conda in Snakemake:

    - Snakemake provides integrated package management via Conda to define isolated software environments per rule

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    - Directive: **conda**
        - Value: path to the environment file relative to the rule's snakefile

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    conda:
        '../envs/py.yaml'
    shell:
        'mv {input} {output}'
```

Being reproducible with Snakemake and Conda

- Using conda in Snakemake:

  - Snakemake provides integrated package management via Conda to define isolated software environments per rule

  - Directive: **conda**
    - Value: path to the environment file relative to the rule's snakefile

  - Execution parameter:

    - v7 and before: --use-conda

    - v8+: --software-deployment-method or --sdm (shorthand version)

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rule rename_file:
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    conda:
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    shell:
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```

```
snakemake --cores 1 --use-conda results/renamed_file.txt
```

```
snakemake --cores 1 --sdm conda
    results/renamed_file.txt
```

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- Using Docker in Snakemake:

  - Snakemake provides a container integration: it can automatically spawn a container created from a given image

  - Directive: **container**
    - Value: URL/path to the image location
    - Handles Docker and Apptainer images
    - **Global** <u>**OR**</u> **rule-specific**

```
container: 'docker://geertvangeest/deseq2:v1'

rule rename_file:
    input:
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  - Execution parameter
    - v7 and before: --use-singularity
    - V8+: --sdm apptainer

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snakemake –cores 1 —sdm apptainer results/renamed_file.txt
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- Using Docker in Snakemake:

  - Snakemake provides a container integration: it can automatically spawn a container created from a given image

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  - Execution parameter
    - v7 and before: --use-singularity
    - V8+: --sdm apptainer

  - Can be combined with conda --sdm conda apptainer
    - Pull the image
    - Create the conda env from **within the container**

  - Containerisation of Conda-based workflows

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```
snakemake –cores 1 —use-singularity results/renamed_file.txt
snakemake –cores 1 —sdm apptainer results/renamed_file.txt
```

```
snakemake –cores 1 —sdm conda apptainer results/renamed_file.txt
```

```
snakemake --cores 1 —containerize > Dockerfile
```

Snakemake environments

- Question 6

What is the best setting for Snakemake environments?

- Use package and container managers!

- Same as Snakefile and config files: split things reasonably
  - 1 .smk file ≈ 1 'thematic' module ≈ 1 environment

- Always check for version conflicts

Exercises

- **Through the day:**
  - Develop a simple RNAseq analysis workflow, from reads (fastq files) to Differentially Expressed Genes (DEG)

- **For this session:**
  - Create and use an input function
  - Run R and Python scripts
  - Deploy a conda environment
  - Deploy a Docker/Singularity container

Conclusion

- Snakemake helps with reproducibility:
    - OS, language, software, versions, parameters control via Conda and containers
        - Avoid installation problems!
    - Readability: written in Python, has a well-defined structure
    - Availability: easy to share via WorkflowHub, [Snakemake workflow catalog](#) or git
    - **Every command run by Snakemake is saved!**

- And it has many uses:
    - Easily deployable/executable, **locally** or **remotely**
    - Scalable, up to thousands of jobs
        - Easy to parallelise
    - Snakemake can do a lot for you!
    - Beautiful DAG in one command, no more powerpoint or Photoshop!