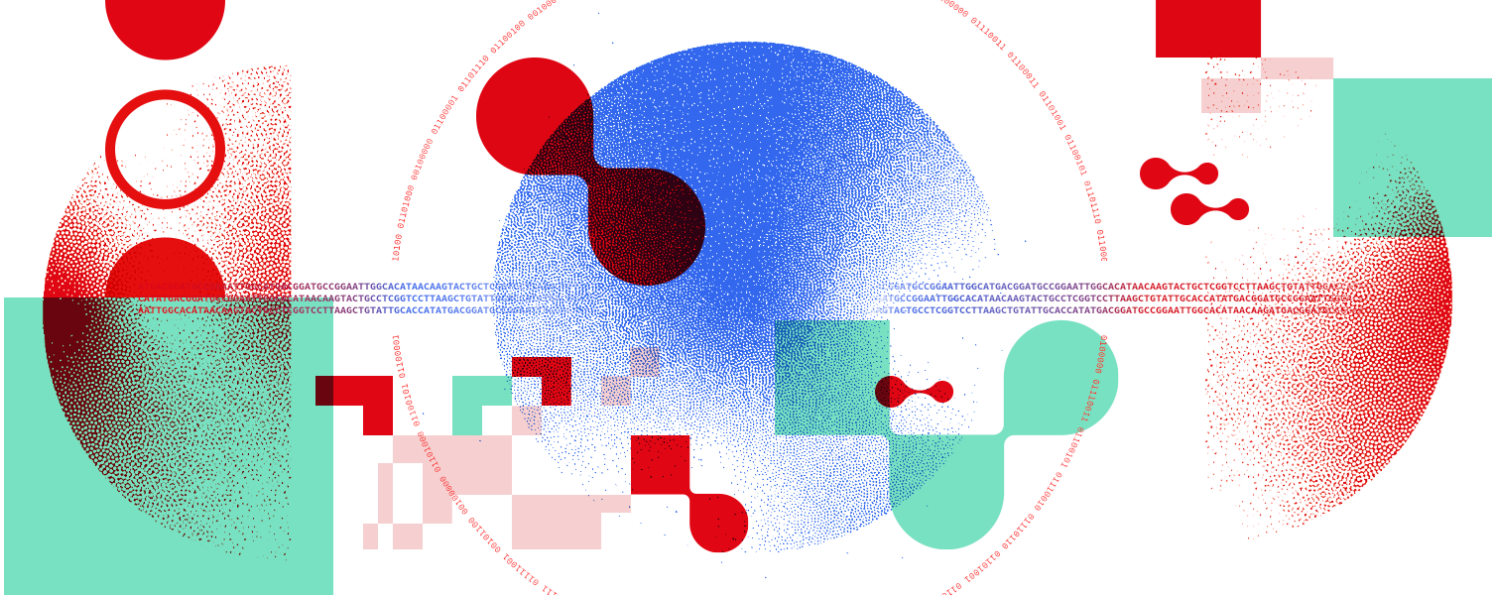

Snakemake for reproducible research

Making a more general-purpose Snakemake workflow

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SIB Swiss Institute of Bioinformatics

Pop quiz

```
rule rename_file:
    input:
        rules.create_file.output
    output:
        'results/renamed_file.txt'
    shell:
        'mv {input} {output}'
```

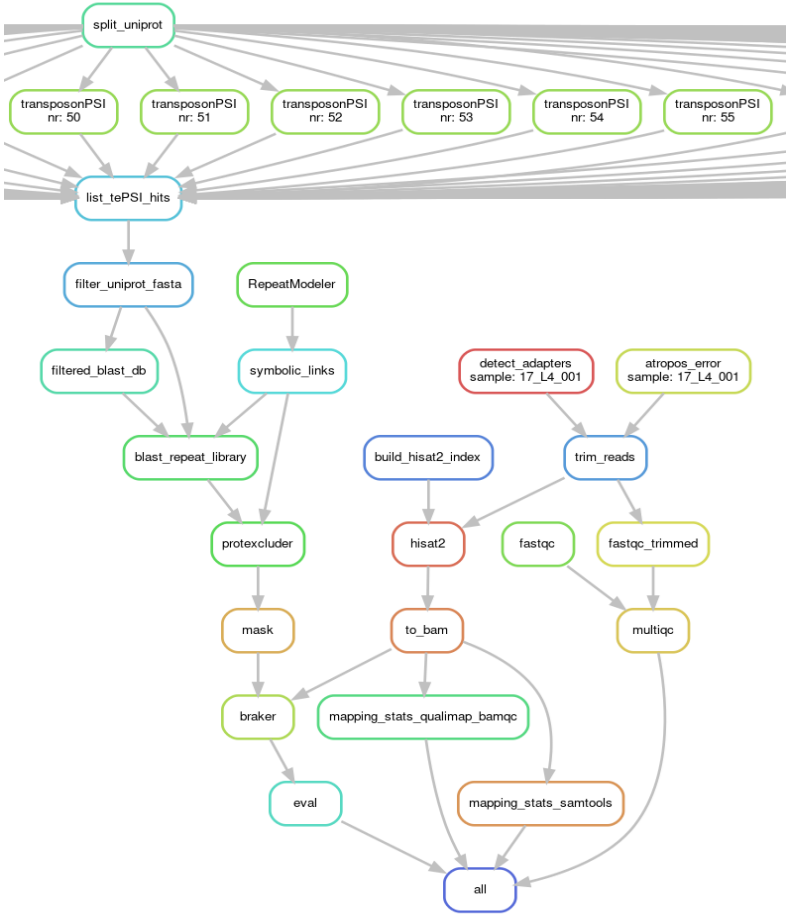
Pop quiz

- Snakemake keyword
- Rule name (user-defined)
- Snakemake directives
- Directives values:
  - Object
  - String (file path)
  - Instruction (command)
  - Numeric values (seen later)
- Mystery syntax?

```
rule rename_file:
    input:
        rules.create_file.output
    output:
        'results/renamed_file.txt'
    shell:
        'mv {input} {output}'
```

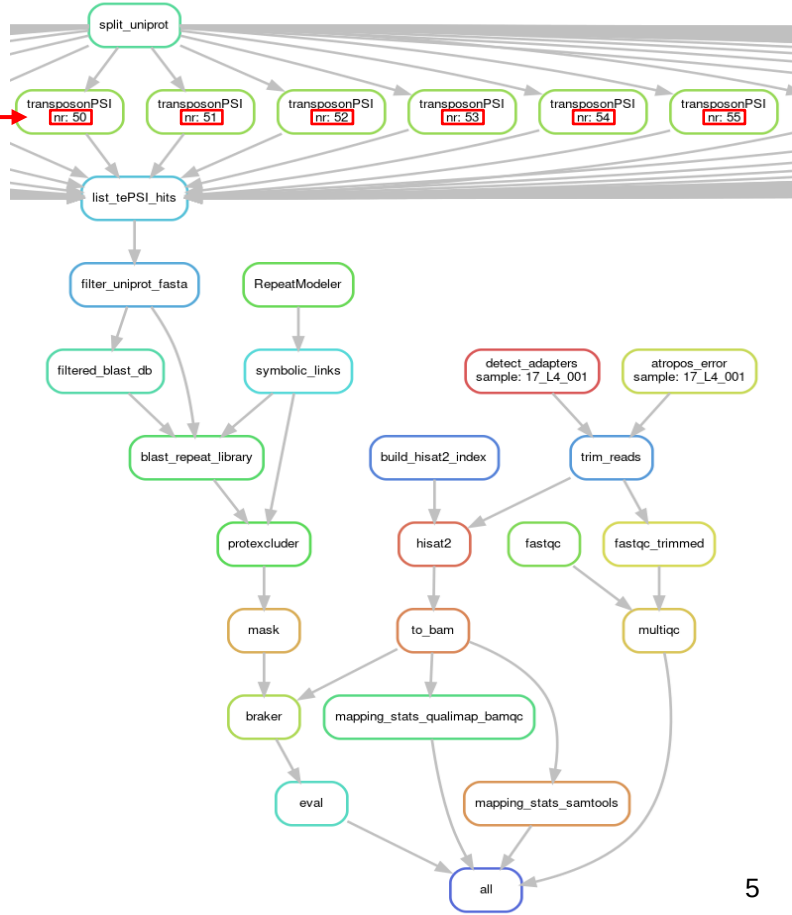
Building a Directed Acyclic Graph (DAG)

- Snakemake determines which jobs to run to produce desired outputs



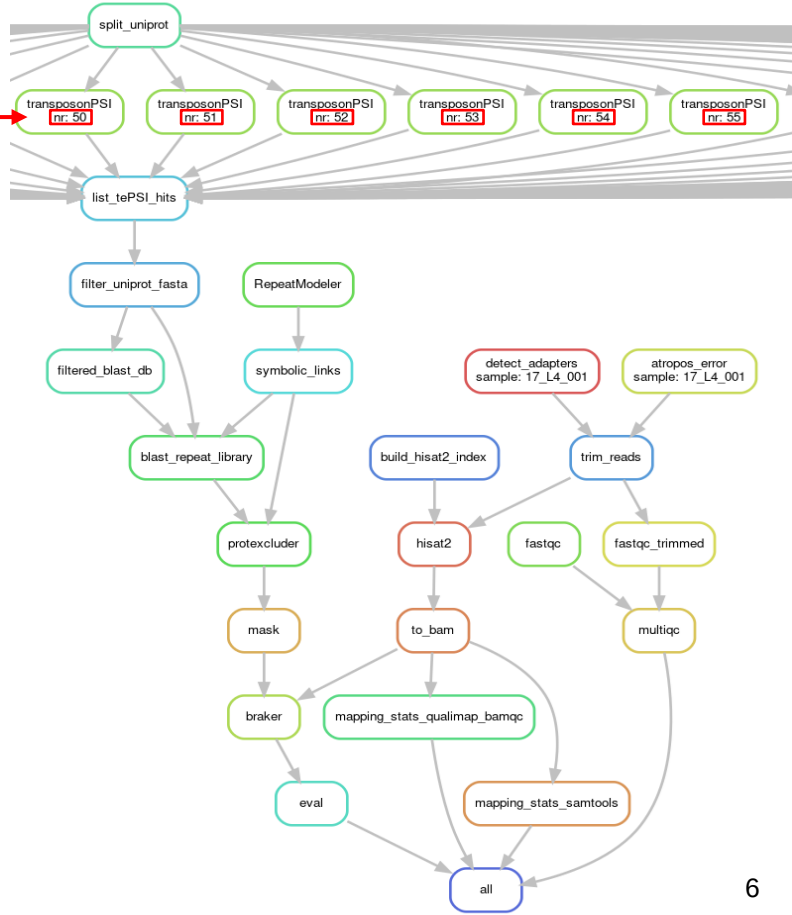
Building a Directed Acyclic Graph (DAG)

- Snakemake determines which jobs to run to produce desired outputs
- Rule can appear more than once, with different wildcards
  - 1 rule + 1 wildcard values = 1 job
- Arrows = dependency between jobs
  - Snakemake runs jobs in any order that doesn't break dependency



Building a Directed Acyclic Graph (DAG)

- Snakemake determines which jobs to run to produce desired outputs
- Rule can appear more than once, with different wildcards
  - 1 rule + 1 wildcard values = 1 job
- Arrows = dependency between jobs
  - Snakemake runs jobs in any order that doesn't break dependency
- DAG = work list, ≠ flowchart
  - No if/else decisions or loops
  - Snakemake runs every job in the DAG exactly once
- DAG ≠ checking shell directives
  - Shell commands are tested during execution
    - Works? Produces expected outputs?



What is a DAG useful?

- Skip parts of the DAG to avoid recomputing → Save time and resources (CPU, memory, energy, money)
- Change/add inputs to existing analyses without re-running everything
- Resume running a workflow that failed part-way

What could we improve?

What could we improve?

- Using hard-coded file paths
- Having multiple inputs/outputs per rule
- (Checking Snakemake behaviour)

What could we improve?

- Using hard-coded file paths → Placeholders and wildcards
- Having multiple inputs/outputs per rule → Numbered/named inputs/outputs
- (Checking Snakemake behaviour) → (Log files, benchmarks)

Avoiding hard-coded filepaths: placeholders

- Placeholder:
  - A person or thing that occupies the position or place of another person or thing
  - A symbol in a mathematical or logical expression that may be replaced by the name of any element of a set

(From the Merriam-Webster dictionary)

Avoiding hard-coded filepaths: placeholders

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv data/test.txt results/renamed_test.txt'
```

Avoiding hard-coded filepaths: placeholders

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv data/test.txt results/renamed_test.txt'
```

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv {input} {output}'
```

Avoiding hard-coded filepaths: placeholders

- {input} and {output} are placeholders
- Used in shell directive
- Similar to python f-string
- Snakemake will replace them with appropriate values before running the command
- Many directives can use placeholders: {log}, {benchmark}, {params}…

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv {input} {output}'
```

Making more general-purpose rules: wildcards

- Wildcards ≈ "variables" automatically inferred by Snakemake

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rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv {input} {output}'
```

Defined paths

Making more general-purpose rules: wildcards

- Wildcards ≈ "variables" automatically inferred by Snakemake

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv {input} {output}'
```

Defined paths

```
rule rename_file:
    input:
        'data/{file}.tsv'
    output:
        'results/{file}.txt'
    shell:
        'mv {input} {output}'
```

Adaptable paths with wildcards

Making more general-purpose rules: wildcards

- Wildcards ≈ "variables" automatically inferred by Snakemake
- Enclose wildcard name with curly brackets { }

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_test.txt'
    shell:
        'mv {input} {output}'
```

Defined paths

```
rule rename_file:
    input:
        'data/{file}.tsv'
    output:
        'results/{file}.txt'
    shell:
        'mv {input} {output}'
```

Adaptable paths with wildcards

Making more general-purpose rules: wildcards

- Wildcards are "resolved" from the target and propagated to other directives
  - Regular expression matching: .+
    - "1 or more occurrences of any character except newline"
  - Can be constrained

- Using **wildcards forces to ask for output(s)**: Snakemake doesn't guess!
  - Target rules cannot contain wildcards

```
rule rename_file:
    input:
        'data/{file}.txt'
    output:
        'results/renamed_{file}.txt'
    shell:
        'mv {input} {output}'
```

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

```
{file} = "test"
```

```
input: 'data/test.txt'
```

Making more general-purpose rules: wildcards

- Wildcards are "resolved" from the target and propagated to other directives
  - Regular expression matching: `.+`
- Both a workflow and a rule can use multiple wildcards

```
rule rename_file:
    input:
        'data/{file}_{nb}.txt'
    output:
        'results/renamed_{file}_{nb}.txt'
    shell:
        'mv {input} {output}'
```

```
snakemake --cores 1 results/renamed_test_1.txt
```

```
{file} = "test"; {nb} = "1"
```

```
input: 'data/test_1.txt'
```

Making more general-purpose rules: wildcards

- Wildcards are "resolved" from the target and propagated to other directives
  - Regular expression matching: `.+`
- Both a workflow and a rule can use multiple wildcards
- Input and output files do not need to share the same wildcards
- **All outputs, logs… created by a rule must have the same wildcards!**

```
rule rename_file:
    input:
        'data/{file}.txt'
    output:
        'results/renamed_{file}_{nb}.txt'
    shell:
        'mv {input} {output}'
```

```
snakemake --cores 1 results/renamed_test_1.txt
```

```
{file} = "test"; {nb} = "1"
```

```
input: 'data/test.txt'
```

Creating rules with multiple inputs/outputs

- Rules can use multiple inputs/outputs

Creating rules with multiple inputs/outputs

- Rules can use multiple inputs/outputs
  - Separated by a comma
  - Input values are **unpacked** (replaced by a space-separated list)

```
rule gather_files:
    input:
        'data/test1.txt',
        'data/test2.txt'
    output:
        'results/merged_test.txt'
    shell:
        'cat {input} > {output}'
```

```
shell:
    'cat data/test1.txt data/test2.txt > results/merged_test.txt'
```

Creating rules with multiple inputs/outputs

- Rules can use multiple inputs/outputs
  - Separated by a comma
  - Input values are **unpacked** (replaced by a space-separated list)

- Shell can have multiple commands
  - Separated by a semicolon
  - Commands are concatenated

```
rule gather_files:
    input:
        'data/test1.txt',
        'data/test2.txt'
    output:
        'results/merged_test.txt'
    shell:
        'cat {input} > {output}';
        'cat {input} >> {output}'
```

Creating rules with multiple inputs/outputs

- Rules can use multiple inputs/outputs
  - Separated by a comma
  - Input values are **unpacked** (replaced by a space-separated list)

- Shell can have multiple commands
  - ~~Separated by a semicolon~~
  - Commands are concatenated

```
rule gather_files:
    input:
        'data/test1.txt',
        'data/test2.txt'
    output:
        'results/merged_test.txt'
    shell:
        '''
        cat {input} > {output}
        cat {input} >> {output}
        '''
```

Creating rules with multiple inputs/outputs

- Rules can use multiple inputs/outputs
  - Separated by a comma
  - Input values are **unpacked** (replaced by a space-separated list)
- Shell can have multiple commands
  - ~~Separated by a semicolon~~
  - Commands are concatenated
- Inputs can be accessed by their positional index: input[n]
  - **Numbering starts at 0**

```
rule gather_files:
    input:
        'data/test1.txt',
        'data/test2.txt'
    output:
        'results/merged_test.txt'
    shell:
        '''
        cat {input[0]} > {output}
        cat {input[1]} >> {output}
        '''
```

Creating rules with multiple inputs/outputs

- Rules can use multiple inputs/outputs
  - Separated by a comma
  - Input values are **unpacked** (replaced by a space-separated list)
- Shell can have multiple commands
  - ~~Separated by a semicolon~~
  - Commands are concatenated
- Inputs can be accessed by their positional index: `input[n]`
  - **Numbering starts at 0**
- Named inputs can be accessed by their names: `input.input_name`

```
rule gather_files:
    input:
        file_1='data/test1.txt',
        file_2='data/test2.txt'
    output:
        'results/merged_test.txt'
    shell:
        '''
        cat {input.file_1} > {output}
        cat {input.file_2} >> {output}
        '''
```

Creating rules with multiple inputs/outputs

- Outputs work like inputs
  - Separated by ','
  - Can be named
  - Can be accessed by positional index or by name
- All outputs need to be created or the job will fail

```
rule gather_files:
    input:
        file_1='data/test1.tsv',
        file_2='data/test2.tsv'
    output:
        copy_1='results/copied_test1.txt',
        copy_2='results/copied_test2.txt'
    shell:
        '''
        cat {input.file_1} > {output.copy_1}
        cat {input.file_2} > {output.copy_2}
        '''
```

```
snakemake --cores 1 results/first_step_1.txt
```

```
'results/first_step_1.txt', 'results/first_step_2.txt'
```

Checking Snakemake behaviour

- Producing log files
- Benchmarking rules

Checking Snakemake behaviour: log files

- 'log' is a directive; its value is a path to a log file for one rule
  - Can be accessed with a placeholder in shell: {log}

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    log:
        'logs/renaming.log'
    shell:
        'mv {input} {output} 2> {log}'
```

Checking Snakemake behaviour: log files

- 'log' is a directive; its value is a path to a log file for one rule
  - Can be accessed with a placeholder in shell: {log}

- You need to manually redirect messages to logs, but Snakemake automatically creates the folder path

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    log:
        'logs/renaming.log'
    shell:
        'mv {input} {output} 2> {log}'
```

Checking Snakemake behaviour: log files

- 'log' is a directive; its value is a path to a log file for one rule
  - Can be accessed with a placeholder in shell: {log}
- You need to manually redirect messages to logs, but Snakemake automatically creates the folder path
- Log files must have the **same wildcards as the output**!
- Good practice: put all logs in same folder

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    log:
        'logs/renaming.log'
    shell:
        'mv {input} {output} 2> {log}'
```

Checking Snakemake behaviour: benchmarks

- 'benchmark' is a directive; its value is a path to a benchmark results file for a rule

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    benchmark:
        'benchmarks/renaming.txt'
    shell:
        'mv {input} {output}'
```

Checking Snakemake behaviour: benchmarks

- 'benchmark' is a directive; its value is a path to a benchmark results file for a rule
- Snakemake will measure **runtime** and **memory usage** for the rule and save it to the file

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    benchmark:
        'benchmarks/renaming.txt'
    shell:
        'mv {input} {output}'
```

Checking Snakemake behaviour: benchmarks

- 'benchmark' is a directive; its value is a path to a benchmark results file for a rule
- Snakemake will measure **runtime** and **memory usage** for the rule and save it to the file
- Benchmark files must have the **same wildcards as the output**!
- Best practice: put all benchmarks in same folder

```
rule rename_file:
    input:
        'data/test.txt'
    output:
        'results/renamed_file.txt'
    benchmark:
        'benchmarks/renaming.txt'
    shell:
        'mv {input} {output}'
```

Exercises

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

- For this session:
  - Use placeholders and wildcards
  - Use multiple inputs and outputs
  - (Check workflow behaviour)
  - Visualise a DAG