A bioinformatic pipeline for GWAS

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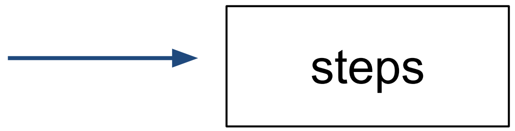
bioinformatic analysis

- typical bioinformatic analyses involve a **series of data transformations / operations** (e.g. joining paired-end reads, assembling reads into longer sequences, aligning sequences to a reference genome, calling variants etc.)



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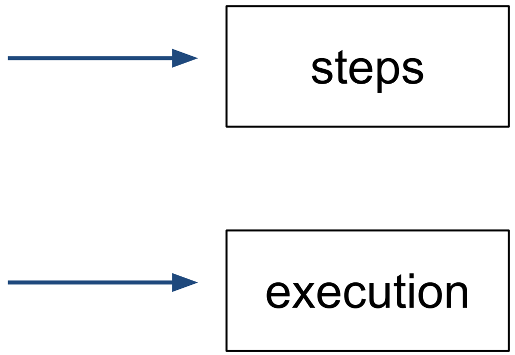

bioinformatic analysis

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- to be run **sequentially** or **in parallel** on one or **multiple samples** / input files $\rightarrow$ execution

✓ this calls for a **structured** / organized **pipeline** / workflow of analysis

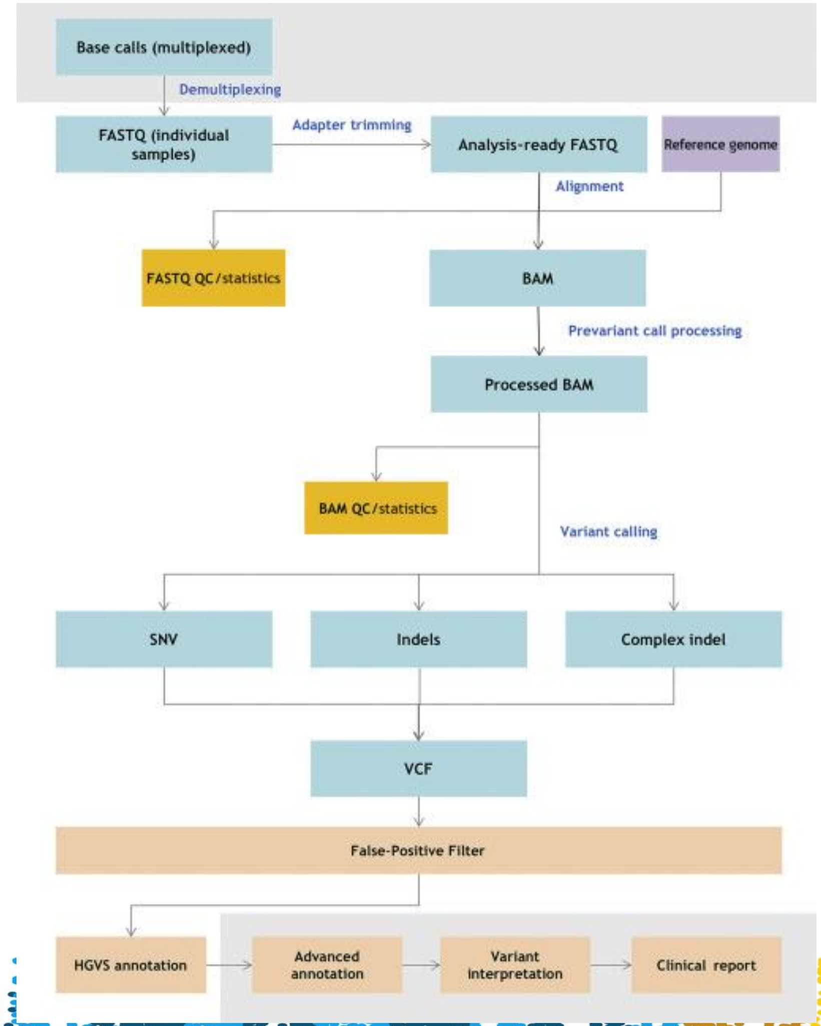
bioinformatic analysis

- large datasets
- embarrassing parallelization (hundreds of jobs)
- many different tools, scripts, languages (dependencies) → difficult to maintain and to update, almost impossible to make it portable

✓ this calls for a **structured** / organized **pipeline** / workflow of analysis

bioinformatic pipelines

- **chaining** together multiple consecutive **steps** (processes) of a data analysis problem/project



bioinformatic pipelines

(Roy et al, 2018)

bioinformatic pipelines

- **chaining** together multiple consecutive **steps** of a data analysis problem/project
- advantages**:**
    - **reproducibility**
    - **modularity**
    - **parallelization**
    - **scalability**
    - **portability**
    - **usability**
    - **automation**

bioinformatic pipelines - reproducibility

Reproducibility: quantum mechanics (probabilistic world) → average (statistical) reproducibility (besides, different environments / different labs → different results are expected); this is true also in bioinformatics (same pipeline, same data, different machines → different results are possible: underlying variance in the libraries, floats, machines -e,g, Mac cs Linux- and so on …)

- https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0080278;
- https://www.nature.com/articles/nbt.3820

bioinformatic pipelines - predecessors

- stand-alone **scripts**: e.g. Unix shell, Python etc.
  - ✓ features: variables, conditional logic
  - limitations: no support for dependencies and reentrancy (e.g. concatenated steps, adding input samples, updating parameters/resources, recovering from local failures, run/start intermediate steps etc.)

bioinformatic pipelines - predecessors

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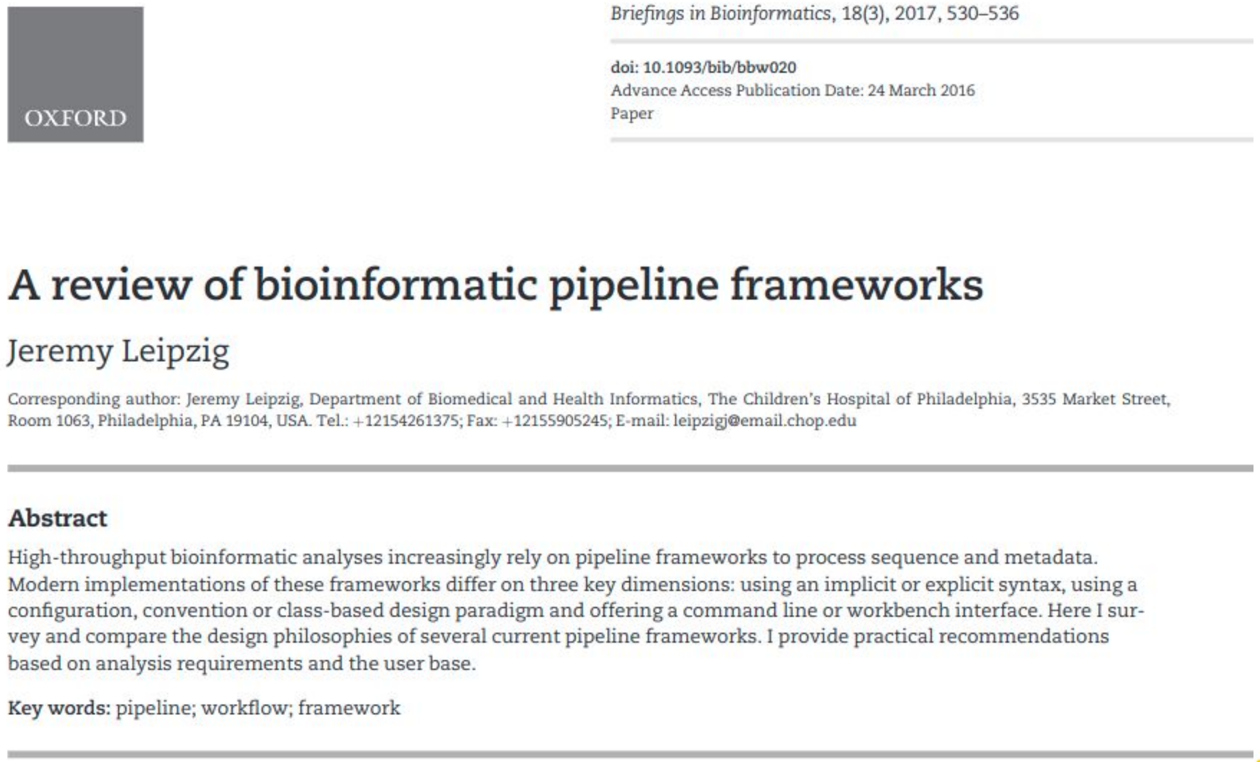
- **Make**: compilation / build automation tool
  - ✓ use an instruction file (Makefile) to generate a target (e.g. compiled software)
  - ✓ features: wildcards, dependency tree
  - limitations: not designed for scientific pipelines, no direct support for distributed/parallel computing, insufficient flexibility for complex parameters, input data, execution logic etc.

bioinformatic pipelines - available frameworks

- pipeline execution engines / workflow management systems
  - **Snakemake:** https://snakemake.readthedocs.io/en/stable/
  - **Nextflow:** https://www.nextflow.io/
    - Pipeline for GWAS? (under construction)
  - **BigDataScript:** https://pcingola.github.io/BigDataScript/
  - **Pipengine:** https://github.com/fstrozzi/bioruby-pipengine

bioinformatic pipelines - reading a bit more



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Paper

A review of bioinformatic pipeline frameworks

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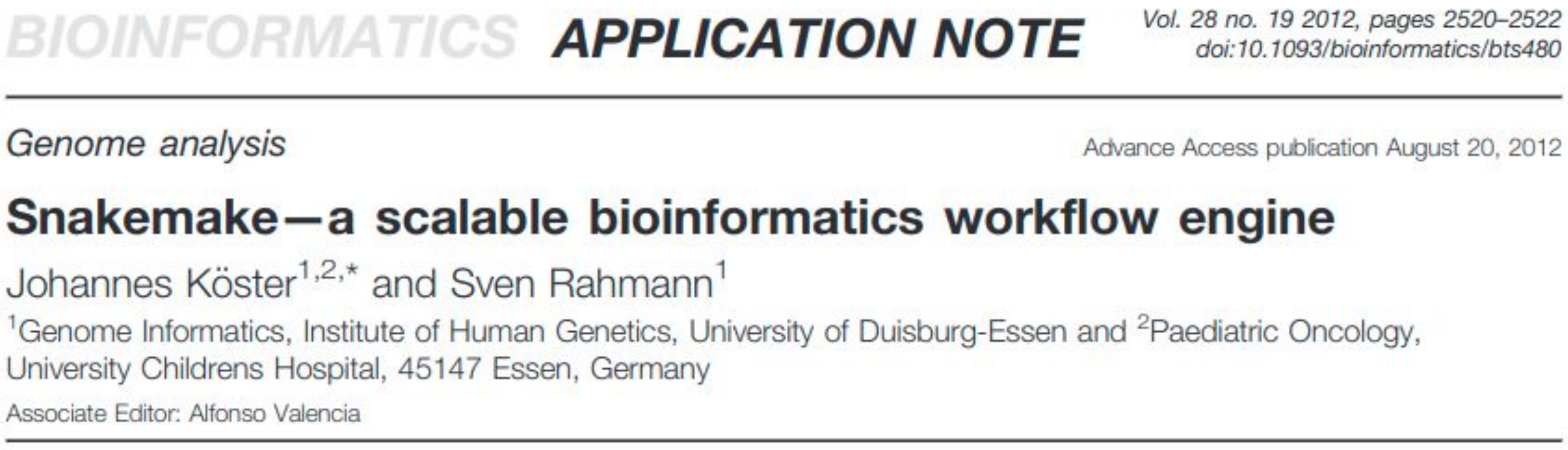
Abstract

High-throughput bioinformatic analyses increasingly rely on pipeline frameworks to process sequence and metadata. Modern implementations of these frameworks differ on three key dimensions: using an implicit or explicit syntax, using a configuration, convention or class-based design paradigm and offering a command line or workbench interface. Here I survey and compare the design philosophies of several current pipeline frameworks. I provide practical recommendations based on analysis requirements and the user base.

Key words: pipeline; workflow; framework

Snakemake

bioinformatic pipelines - Snakemake



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Snakemake—a scalable bioinformatics workflow engine

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- Pythonic variant of GNU Make
- Makefile → **Snakefile**

bioinformatic pipelines - Snakemake

- pipelines are defined in terms of **rules** that define how to create output files from input files:

  input → [rule] → output

- **dependencies** between the rules are determined automatically, creating a DAG (directed acyclic graph) of jobs that can be automatically parallelized

Snakemake - the snakefile

```
## target rule: files that we want to generate
## as final output of the pipeline
rule targets:
    input:
        "snp_sorted.map.gz"

## step 2
## rule to compress the sorted map file
rule gzip:
    input:
        "snp_sorted.map"
    output:
        "snp_sorted.map.gz"
    shell:
        "gzip {input}"

## step 1
## rule to sort the map file
rule sort:
    input:
        "snp.map"
    output:
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    shell:
        "sort -n -k1 {input} > {output}"
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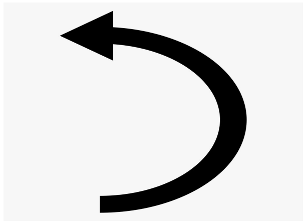

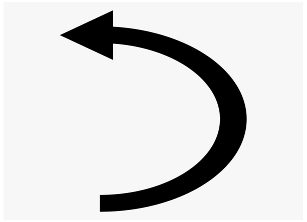

[if exists(snp_sorted.map.gz)] else:

[if snp_sorted.map.gz > snp_sorted.map] else:

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keywords: rule, input, output, shell
wildcards: {}, {input}, {output}

Snakemake - execution

```
snakemake --help

snakemake --dryrun --snakefile example_snakefile
```

Snakemake - execution

```
snakemake --help

snakemake --dryrun --snakefile example_snakefile

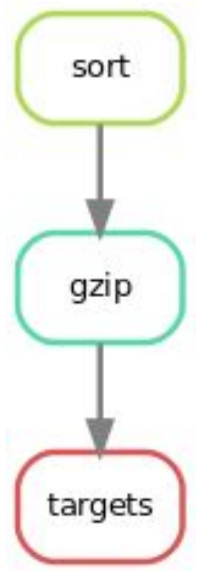
snakemake -n -s example_snakefile
```

[have a look at the log from the dry run]

short option names

Snakemake - the DAG

```
snakemake --dag --dryrun --snakefile
example_snakefile | dot -Tsvg > dag_example.svg
```



Snakemake - execution

```
snakemake --snakefile example_snakefile --cores 1
```

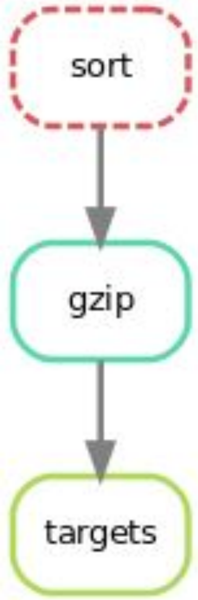
- true run
- check output

Snakemake - execution

```
snakemake --snakefile example_snakefile -c 1
```

- actual run
- check output

⚠️ what happens if you unzip the sorted file? (try first the dry run, then the actual run)



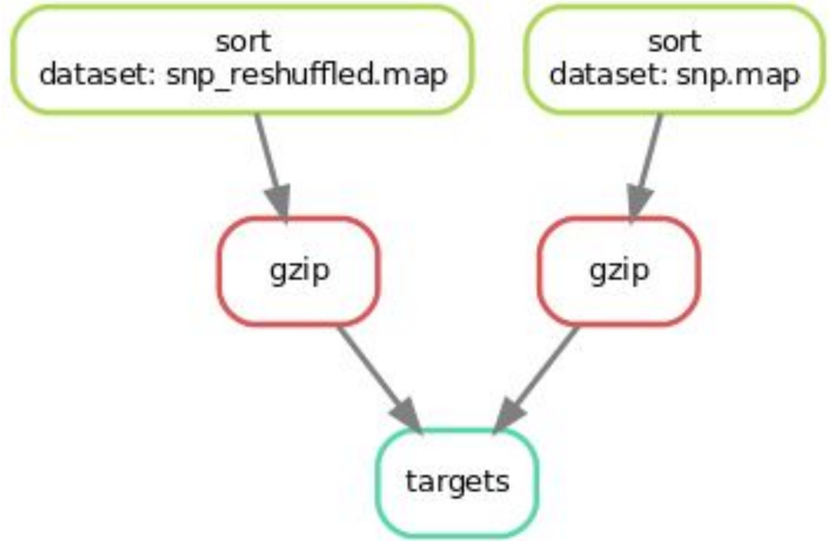
Snakemake - multiple samples

```
## samples
DATASETS = ['snp.map', 'snp_reshuffled.map']


## target rule: files that we want to generate
rule targets:
    input:
        expand("{dataset}.sorted.gz", dataset=DATASETS)


## step 2 - rule to compress the sorted map file
rule gzip:
    input:
        "{dataset}.sorted"
    output:
        "{dataset}.sorted.gz"
    shell:
        "gzip {input}"


## step 1 - rule to sort the map file
rule sort:
    input:
        "{dataset}"
    output:
        "{dataset}.sorted"
    shell:
        "sort -n -k1 {input} > {output}"
```



Snakemake - multiple samples

```
snakemake -n --snakefile example_snakefile_2 | dot -Tsvg >
dag_example.svg

snakemake --snakefile example_snakefile_2 --cores 1
```

- dry run
- actual run

The GWAS pipeline

GWAS pipeline - the atomized steps

1. download and prepare the data
2. data preprocessing and filtering
3. imputation of missing genotypes
4. GWAS

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⚠️ each folder contains a bash script to remove intermediate and output files (reset the step): **clean_folder.sh**

⚠️ set the correct paths to resources in the bash scripts (e.g. **Plink**, **Beagle** etc.)