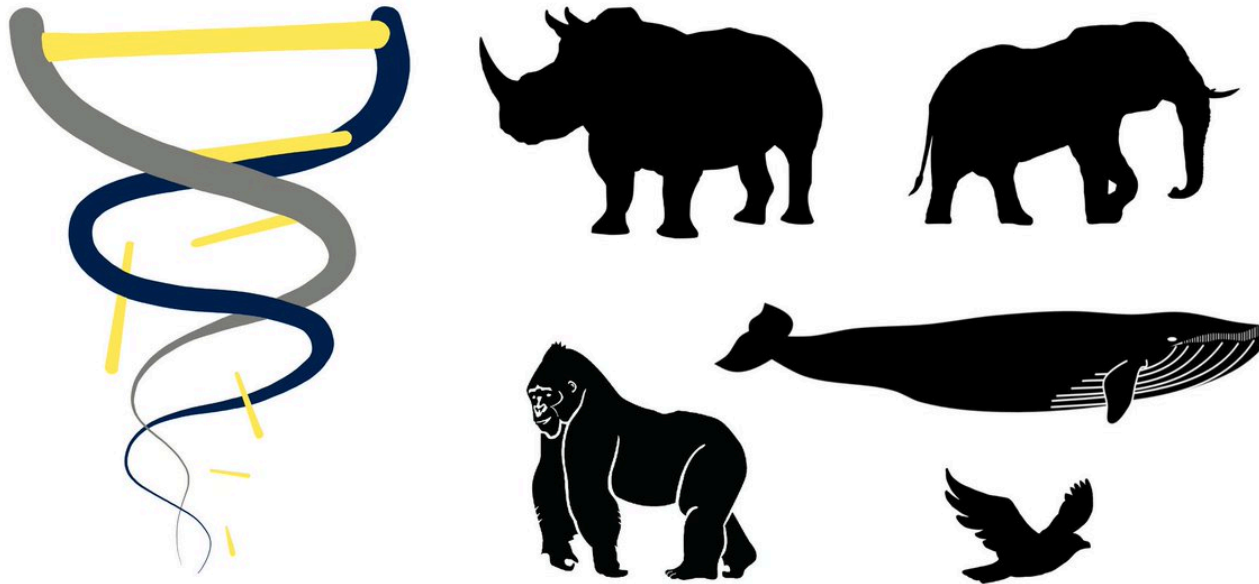


An Example Snakemake Workflow

The GenErode pipeline



<https://github.com/NBISweden/GenErode>

Kutschera et al. 2022 (BMC Bioinformatics)

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Sumatran rhinoceros (*Dicerorhinus sumatrensis*), critically endangered

- Data processing from fastq files to BAM & VCF files plus downstream population genomics analyses

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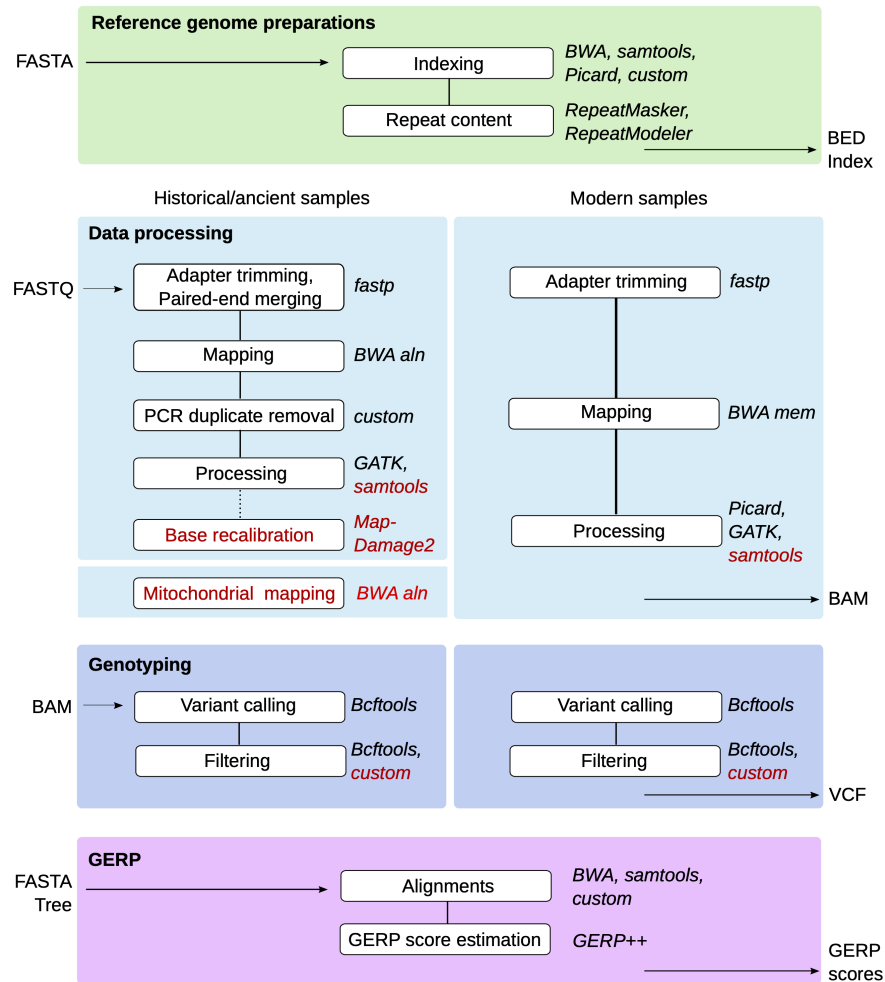
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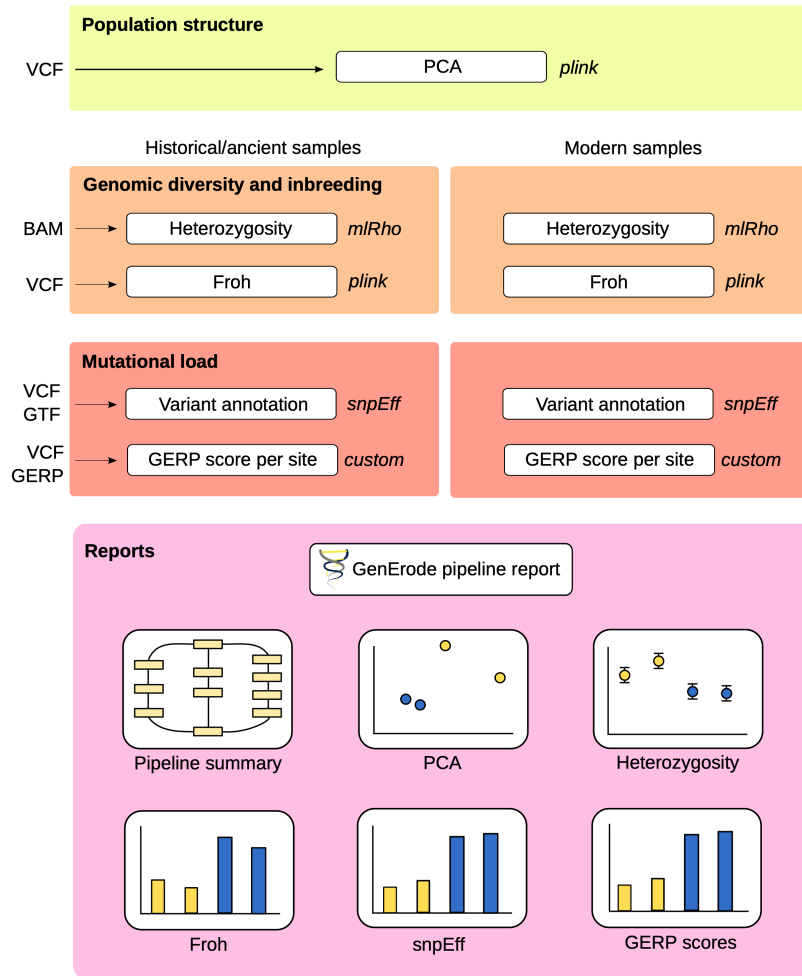
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- Whole-genome resequencing data from historical/ancient samples needs special processing as DNA degrades over time
- Some analyses or filtering steps are run separately for modern and historical samples, or only for historical samples

Analysis Tracks of the Workflow



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 - `include` of rule Snakefiles
 - `all` rule collecting output files produced by the different rule Snakefiles
 - Python and bash code to generate and edit the pipeline report with `snakemake --report`
- Cluster execution with slurm (e.g. UPPMAX):
 - `config/slurm/` folder with configuration files for the slurm profile and for the deprecated cluster configuration (`--cluster` still exists in Snakemake v7 but not in v8 anymore)

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 - Sample IDs, readgroup IDs, sequencing technology, paths to fastq files
- Example `historical_samples.txt` file (whitespace-separated)

samplename_index_lane	readgroup_id	readgroup_platform	path_to_R1_fastq	path_to_R2_fastq
VK01_01_L2	BHYOX3ALTH.L2.01	illumina	data/S1/P01_2.R1.fq.gz	data/S1/P01_2.R2.fq.gz
VK01_02_L2	BHYOX3ALTH.L2.02	illumina	data/S1/P02_2.R1.fq.gz	data/S1/P02_2.R2.fq.gz

- see `config/historical_samples_paths.txt` and `config/modern_samples_paths.txt`

The Workflow Structure

- Config file `config.yaml` (to be edited by users, placed in `config/`)
 - Paths to input data and metadata tables
 - Selection of analysis steps to be run
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 - Lists with samples for optional analyses

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```
#####
# 1) Full path to reference genome assembly.
# Reference genome has to be checked for short and concise FASTA
# headers without special characters and has to be uncompressed.
# The file name will be reused by the pipeline and can have the file
# name extensions *.fasta, *.fa or *.fna.
ref_path: ""
#####

#####
# 2) Relative paths (from the main snakemake directory) to metadata
# files with sample information.
# Example files can be found in "config/"
historical_samples: "" # leave empty (") if not run for historical samples.
modern_samples: "" # leave empty (") if not run for modern samples.
#####
```

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Step 1: Use booleans in the config file (`config/config.yaml`) as on/off switches

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# FastQC on raw reads, adapter and quality trimming (incl. read merging  
# for historical samples) using fastp, FastQC on trimmed reads.  
# Adapter sequences are automatically detected.  
# Automatic detection of NovaSeq or NextSeq samples and activation of  
# poly-G tail trimming.  
fastq_processing: True  
  
[...]  
#####  
  
#####  
# Map historical and modern reads to reference genome assembly (specified above).  
mapping: False  
#####
```

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# for historical samples) using fastp, FastQC on trimmed reads.
# Adapter sequences are automatically detected.
# Automatic detection of NovaSeq or NextSeq samples and activation of
# poly-G tail trimming.
fastq_processing: True

# Minimum read length.
# Historical samples (after trimming and read merging)
hist_readlength: "30" # recommended setting: 30 bp

# Modern samples (after trimming)
mod_readlength: "30"
#####
```

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- the `include` variable to attach the rule Snakefiles corresponding to the analysis steps that were set to `True` in the config file,

```
if config["fastq_processing"]:  
    include: "workflow/rules/1.1_fastq_processing.smk"
```

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- and the rule `all` that takes the output files from the list `all_outputs` as input

```
rule all:  
    input: all_outputs,
```

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- The rule Snakefile (`workflow/rules/1.1_fastq_processing.smk`) contains some Python code to add its final output files to the list `all_outputs` in the main `Snakefile`

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```
import os
if os.path.exists(config["historical_samples"]):
    all_outputs.append("data/raw_reads_symlinks/historical/stats/multiqc/multiqc_report.html")
    all_outputs.append("results/historical/trimming/stats/multiqc/multiqc_report.html")

if os.path.exists(config["modern_samples"]):
    all_outputs.append("data/raw_reads_symlinks/modern/stats/multiqc/multiqc_report.html")
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    all_outputs.append("data/raw_reads_symlinks/modern/stats/multiqc/multiqc_report.html")
    all_outputs.append("results/modern/trimming/stats/multiqc/multiqc_report.html")
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- `config["historical_samples"]` and `config["modern_samples"]` point to the config file where the paths to metadata files are specified:

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# Example files can be found in "config/"
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- By using the `if` statement to check for the presence of historical or modern metadata files, the workflow can also be run only for historical or only for modern samples

Questions?

