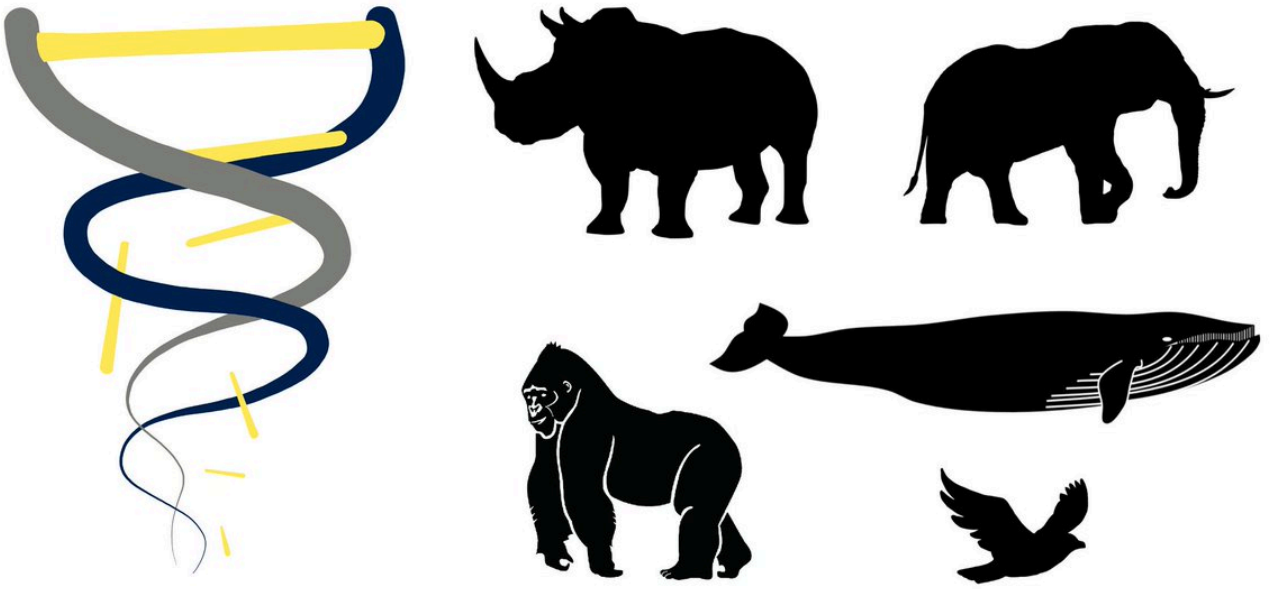
An Example Snakemake Workflow

The GenErode pipeline



https://github.com/NBISweden/GenErode

Kutschera et al. 2022 (BMC Bioinformatics)

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The GenErode pipeline

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Sumatran rhinoceros (*Dicerorhinus sumatrensis*), critically endangered

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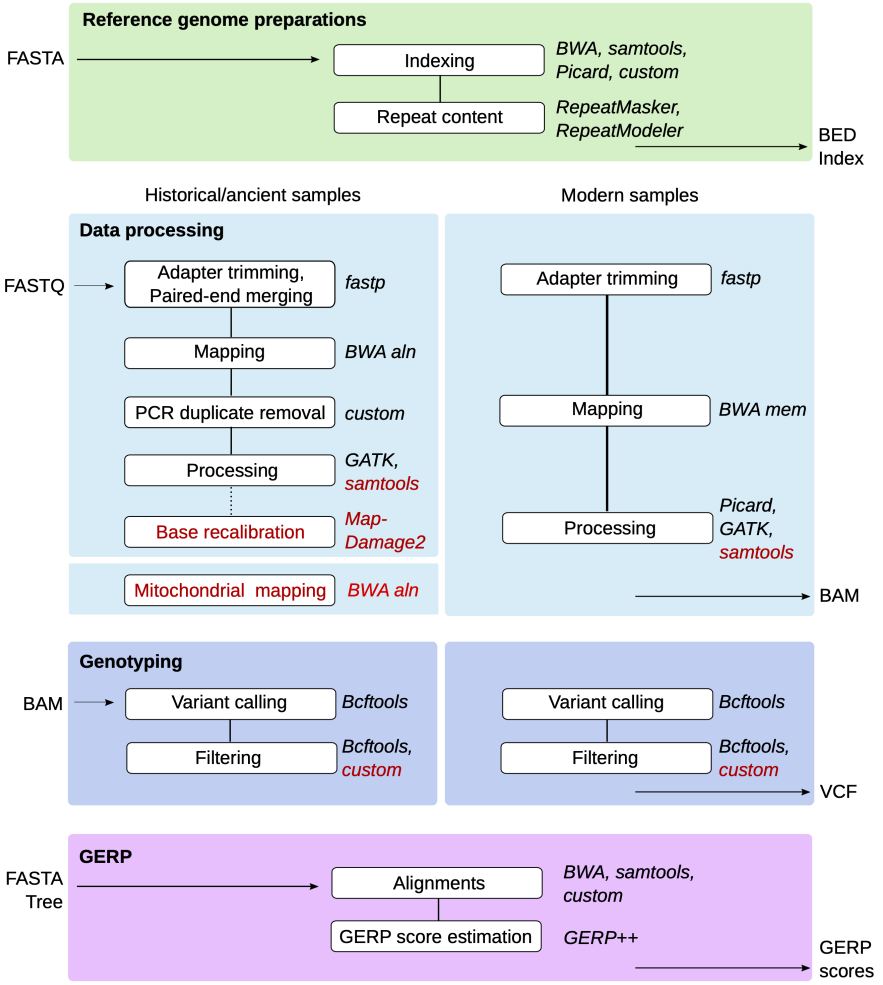
- Historical and modern samples are processed in parallel
- Whole-genome resequencing data from historical/ancient samples needs special processing as DNA degrades over time
- Some analyses or filtering steps are run separately for modern and historical samples, or only for historical samples

Analysis Tracks of the Workflow



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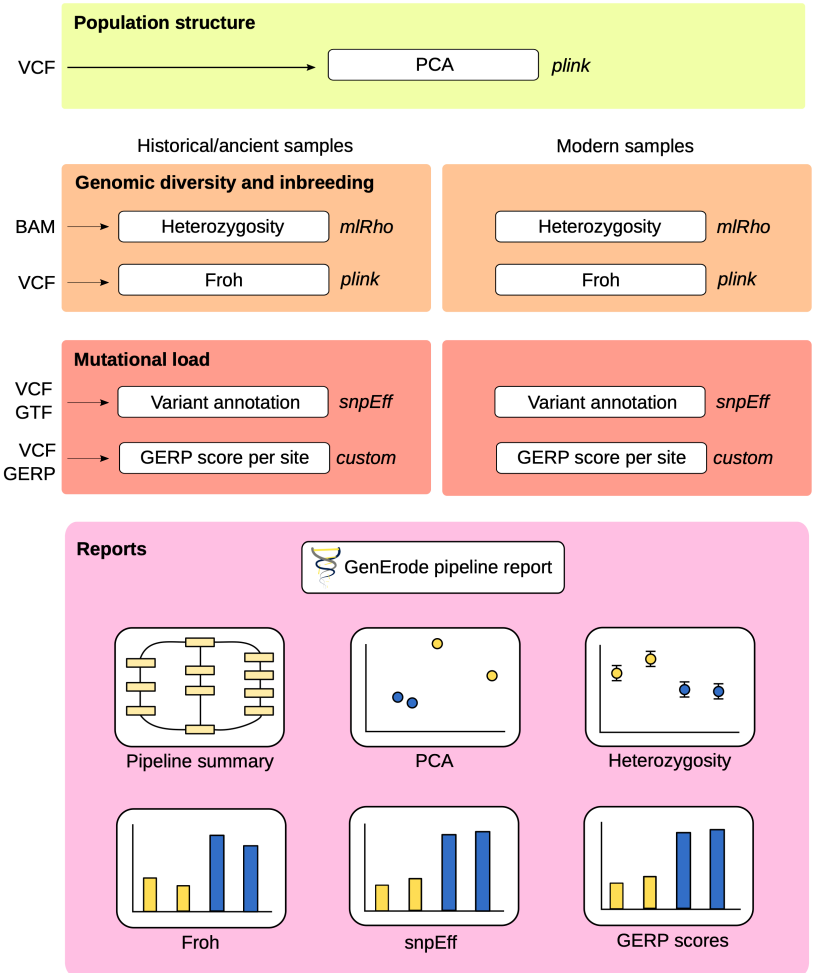

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The Workflow Structure

- Rules with the actual analyses in separate Snakefiles (in `workflow/rules/`)
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- `Snakefile`
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  - `all` rule collecting output files produced by the different rule Snakefiles
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- Cluster execution (e.g. UPPMAX) with slurm:
  - `config/cluster.yaml` file to set up slurm profile

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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
  - Parameters for different rules
  - 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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How to choose which steps to run

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

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#####
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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

[...]
#####

#####
# Map historical and modern reads to reference genome assembly (specified above).
mapping: False
#####
```

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> *For many analysis steps, parameters can be specified in the config file*
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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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```
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")
    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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- 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?