

An Example Snakemake Workflow

The GenErode pipeline

- Developed in a NBIS project with Love Dalén's lab (Centre for Palaeogenetics, SU & NRM)
- Compares population genomics statistics from historical and modern samples of endangered populations



Sumatran rhinoceros (*Dicerorhinus sumatrensis*), critically endangered

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

The GenErode pipeline

- Started at Snakemake version 3.10 (!), current pipeline runs with Snakemake version 5.22
- 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

- Data processing track
 - Repeat element identification
 - Fastq file processing *
 - Optional: mapping to mitochondrial genomes *
 - Mapping to reference genome *
 - BAM file processing *
 - Optional: base quality rescaling for historical samples *
 - Optional: subsampling to target depth *
 - Genotyping
 - Optional: CpG site identification

*Steps of the workflow with different treatment of modern and historical samples

Analysis Tracks of the Workflow

- **BAM file track**
 - mlRho
 - Optional: analyze sex chromosomes separately
 - Optional: remove CpG sites
- **VCF file track**
 - Optional: CpG filtering
 - VCF file processing & merging per dataset
 - Optional: PCA, Runs of homozygosity (ROH), snpEff
- **GERP++ score track**
 - GERP++ score calculation from reference genome and genomes of outgroup species
 - Relative mutational load calculation from GERP++ scores and derived alleles

The Workflow Structure

- Rules with the actual analyses in separate Snakefiles (in `workflow/rules/`)
- Snakefile
 - Python code to create sample and readgroup ID dictionaries & lists
 - From metadata tables and a config file (`config/config.yaml`)
 - `include` of rule Snakefiles
 - `all` rule collecting output files produced by the different rule Snakefiles
- UPPMAX / slurm system:
 - `cluster.yaml` file to set up slurm profile (in `config/`)

The Workflow Structure

- Metadata files (to be created by users, placed in `config/`)
 - Sample IDs, readgroup IDs, sequencing technology, paths to fastq files
 - Separate files for modern and historical samples
- Example `historical_samples.txt` file:

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

- The metadata tables are parsed with Python code (in the main `Snakefile`) to generate sample lists for the workflow

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

```
#####  
# Configuration settings for the genErode pipeline v3.0 #  
# for ancient or historical samples, and modern samples #  
#####  
  
#####  
# 1) Full path to reference genome assembly.  
# Reference genome has to be checked for short and concise fasta  
# headers without special characters.  
# File name extension can be ".fasta" or ".fa".  
ref_path: ""  
#####  
  
#####  
# 2) Relative paths (from the main snakemake directory) to metadata tables of samples.  
# 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.  
#####
```


How to choose which steps to run

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

```
#####  
# FastQC on raw reads, adapter trimming, read merging (historical samples), FastQC on trimmed reads.  
fastq_processing: True  
  
[...]  
#####  
  
#####  
# Map historical and modern reads to reference genome assembly (specified above).  
mapping: False  
#####
```

How to choose which steps to run

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

- For many analysis steps, parameters can be specified in the config file:

```
#####  
# FastQC on raw reads, adapter trimming, read merging (historical samples), FastQC on trimmed reads.  
fastq_processing: True  
  
# Adapter sequences for trimming of historical samples using SeqPrep v1.1 (modified source code) (examples for inserted adapter sequences from Meyer & Kircher 2010).  
# Forward read primer/adaptor sequence to trim historical samples in SeqPrep v1.1 (parameter "-A")  
hist_F_adapter: "AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG"  
# Reverse read primer/adaptor sequence to trim historical samples in SeqPrep v1.1 (parameter "-B").  
# When using double indices, include full adapter length (replacing the index by "N").  
hist_R_adapter: "AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT"  
  
# Fragment of forward adapter sequence used to count occurrence in the first 1 million reads of fastq-files of historical samples  
hist_F_adapter_fragment: "AGATCGGAAGAGCACACGTC"  
# Fragment of reverse adapter sequence used to count occurrence in the first 1 million reads of fastq-files of historical samples  
hist_R_adapter_fragment: "AGATCGGAAGAGCGTCGTGT"  
  
# Minimum read length allowed after trimming.  
# Historical samples (SeqPrep v1.1 with modified source code)  
hist_readlength: "30" # default setting: 30 bp  
  
# Modern samples (trim-galore)  
mod_readlength: "30"  
#####
```

- These parameters can be used in rules with the syntax `config["parameter"]` , e.g.
`config["hist_F_adapter"]`

How to choose which steps to run

Step 2: Use some Python code, `include` and the rule `all` to figure out what the workflow will do

- The main `Snakefile` contains an empty Python list `all_outputs` to collect output files from the included rule Snakefiles,

```
all_outputs = []
```

- 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"  
  
if config["mapping"]:  
    include: "workflow/rules/0.1_reference_genome_preps.smk"  
    include: "workflow/rules/1.1_fastq_processing.smk"  
    include: "workflow/rules/2_mapping.smk"
```

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

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

How to choose which steps to run

Step 2: Use some Python code, `include` and the rule `all` to figure out what the workflow will do

- The rule Snakefile with the analysis step that should be run (e.g. `workflow/rules/1.1_fastq_processing.smk`) contains some Python code to add the output files from its rules to the list `all_outputs` in the main `Snakefile`

```
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/trimmed_merged_reads/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/trimmed_reads/stats/multiqc/multiqc_report.html")
```

- By using the `if` statement to check for the presence of metadata files, the workflow can also be run only for historical or only for modern samples

Questions?

