An Example Snakemake Workflow

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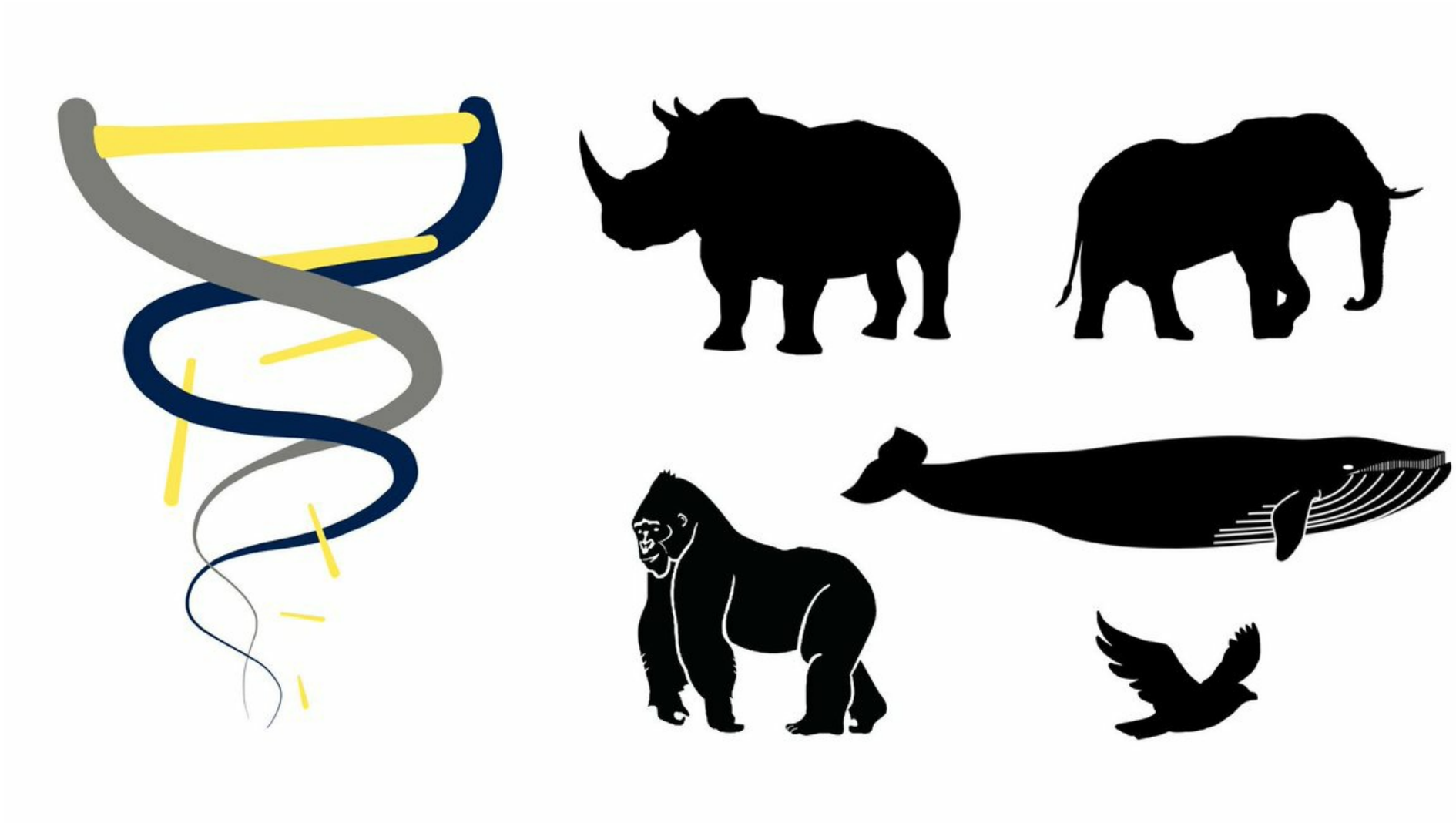
The GenErode pipeline



https://github.com/NBISweden/GenErode

Kutschera et al. 2022 (BMC Bioinformatics)

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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

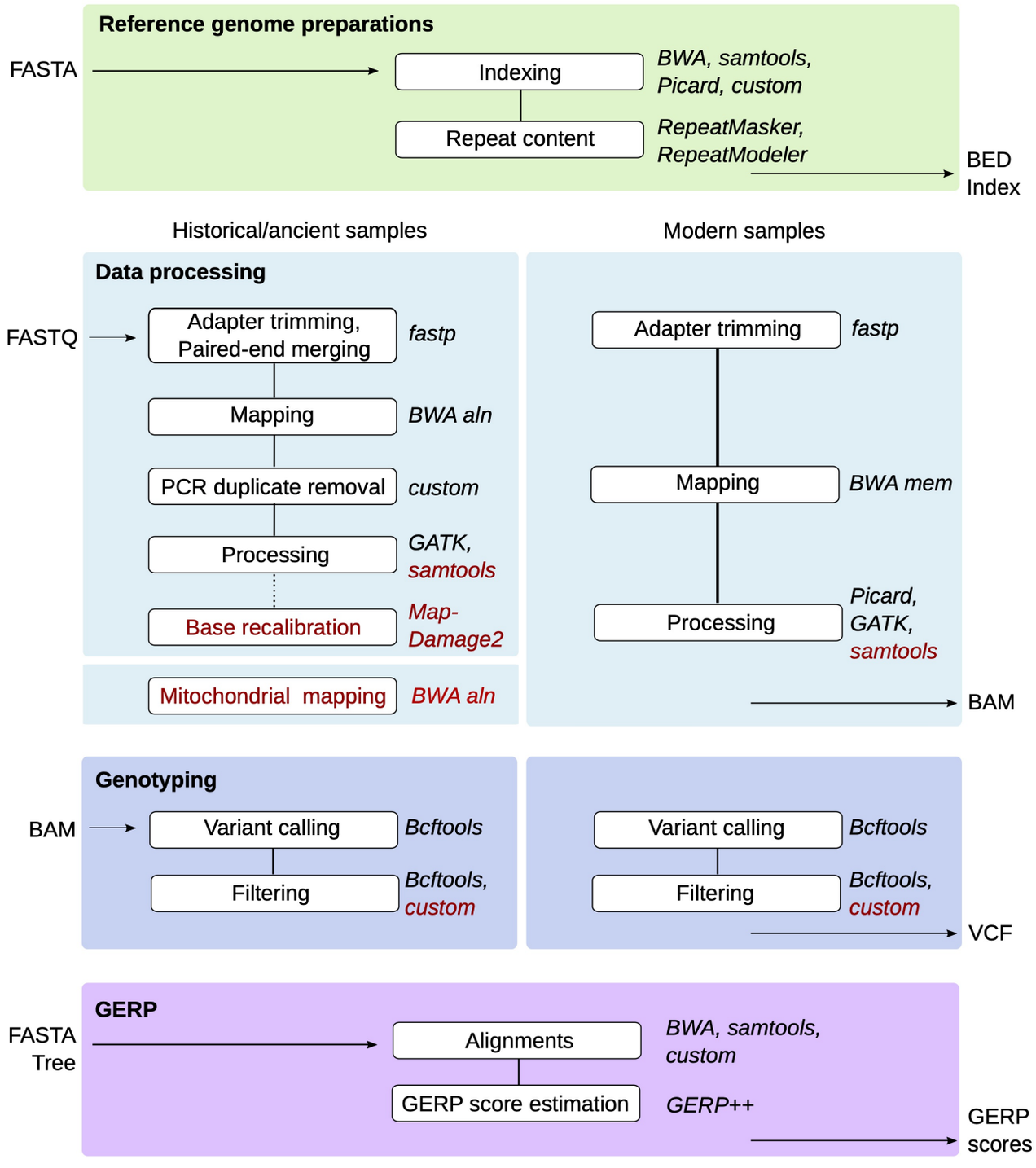
Sumatran rhinoceros (Dicerorhinus sumatrensis), critically endangered

- 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 6.12.1 (latest version: 7.13.0)
- 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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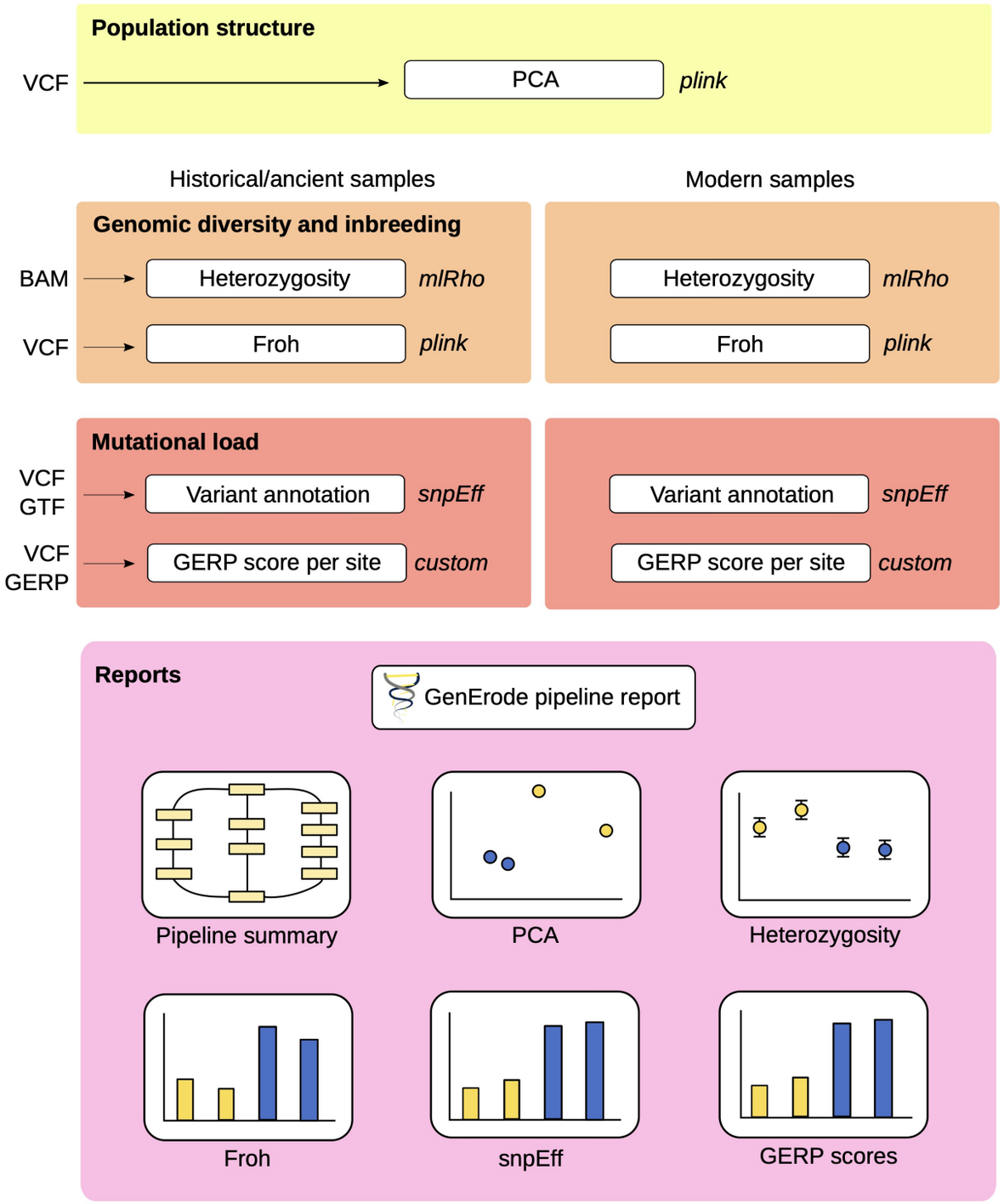
Analysis Tracks of the Workflow



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The Workflow Structure

- Rules with the actual analyses in separate Snakefiles (in `workflow/rules/`)
  - `workflow/rules/common.smk` contains Python code to create sample and readgroup ID dictionaries & lists from metadata tables and the config file

- `Snakefile`
  - `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 (e.g. UPPMAX) with slurm:
  - `config/cluster.yaml` file to set up slurm profile

The Workflow Structure

- Metadata files for historical and modern samples (separately)
  - 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

```
####################################################################
# 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

```
#####
# 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
#####
```

How to choose which steps to run

> For many analysis steps, parameters can be specified in the config file
>
> These parameters can be used in the workflow with the syntax
> `config["parameter_name"]` , e.g. `config["hist_readlength"]`

```
#####
# 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

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

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

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"
```

- 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 (`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`

```
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")
```

- `config["historical_samples"]` and `config["modern_samples"]` point to the config file where the paths to metadata files are specified:

```
#########################################################################
# 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.
#########################################################################
```

How to choose which steps to run

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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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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")
```

- 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?

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