iDiv

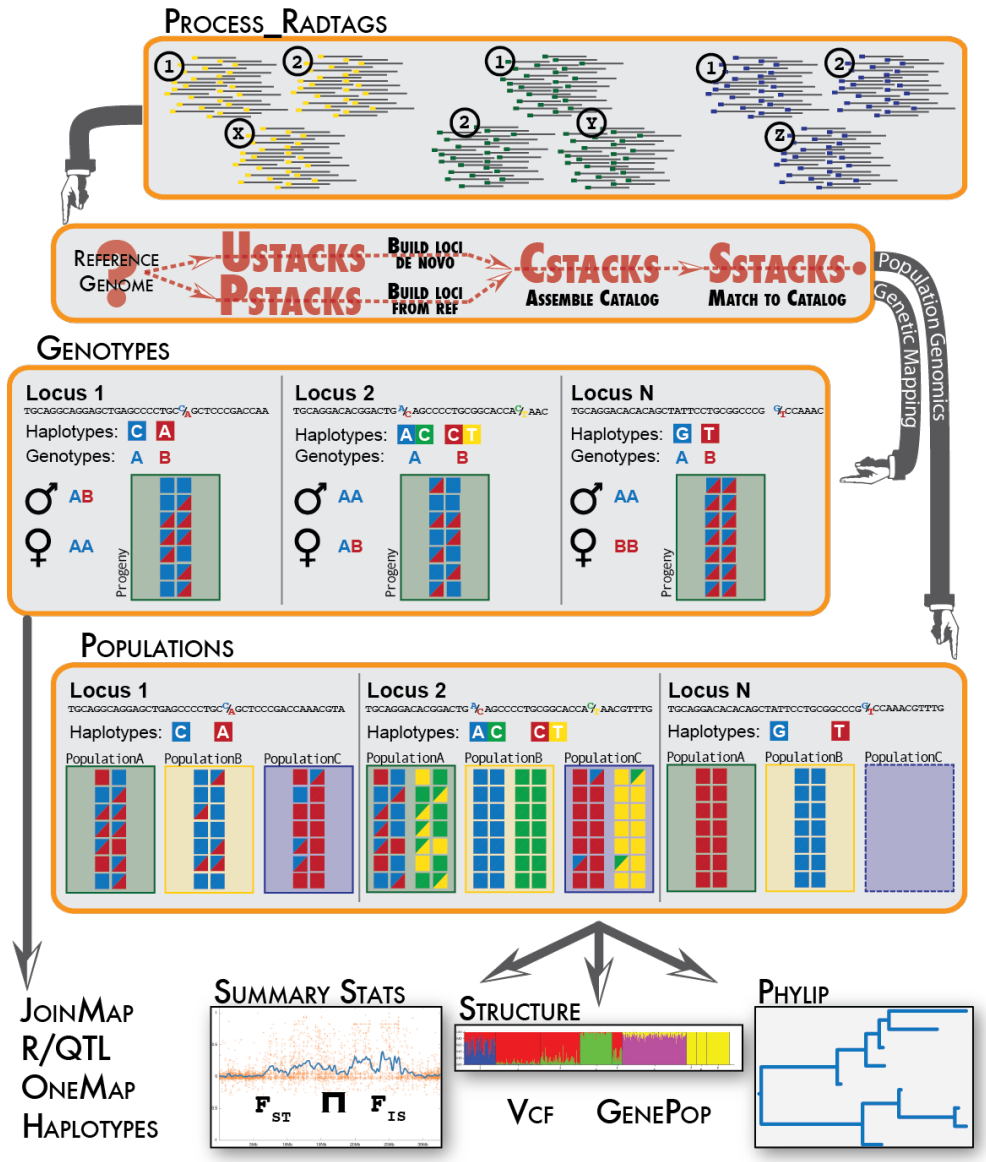
Estimating genetic diversity and population information from short read (ddRAD-seq) type data

03 – denovo_map

Chris Barratt (sDiv / Evolution and Adaptation)

Laura Mendez (Evolution and Adaptation)

Assistant: Dimas Calderon (Evolution and Adaptation)



Rochette, Rivera-Colón & Catchen, 2019 - Mol. Ecology

ddRAD sequencing

ddRADseq Data

HPC cluster (EVE) - UFZ

Stacks v2.61

process_radtags — Clean and demultiplex the data

ustacks — Building loci *de novo* for each sample

cstacks — Creates a *catalog* of all loci across the populations according to sequence similarity

sstacks — Match each sample against the catalog

tsv2bam — Transpose the data to be organized by RAD locus. Paired-end reads are fetched and stored for later use

gstacks — A contig is assembled from pair-end reads and overlapped with the single-end locus. SNP calling

populations — Population level statistics and output in different formats. Possibility for further filtering

Output files for population structure analyses, phylogenetics, demographic history…

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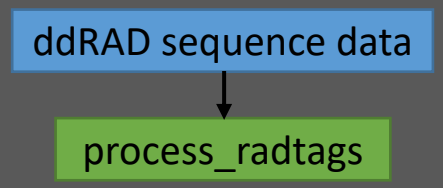
denovo_map.pl

Bioinformatics Workflow



Bioinformatics Workflow

ddRAD sequence data

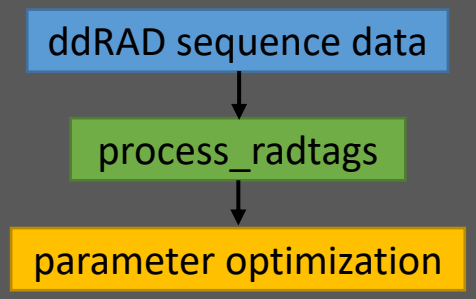
↓

process_radtags

Demultiplexing the data

Sample.1.fq
Sample.rem.1.fq
Sample.2.fq
Sample.rem.2.fq

Bioinformatics Workflow



Bioinformatics Workflow

ddRAD sequence data
↓
process_radtags
↓
parameter optimization

Methods in Ecology and Evolution

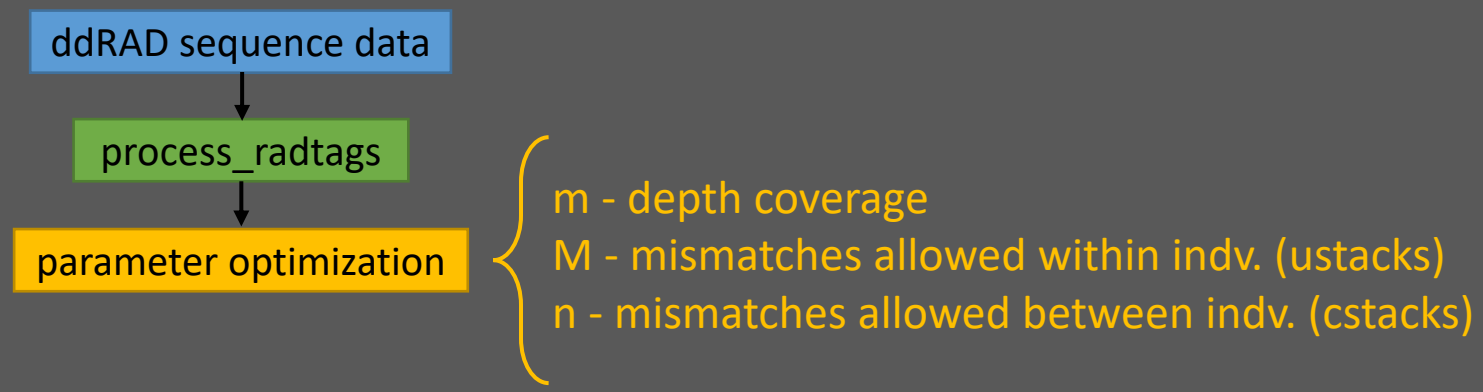
BRITISH ECOLOGICAL SOCIETY

Methods in Ecology and Evolution 2017, **8,** 1360–1373 doi: 10.1111/2041-210X.12775

Lost in parameter space: a road map for STACKS

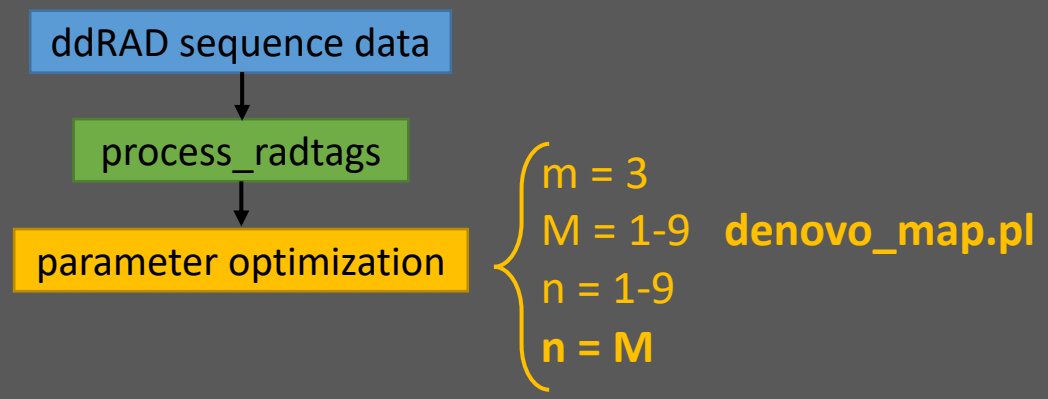
Josephine R. Paris[1], Jamie R. Stevens[1] and Julian M. Catchen*,[2]

Bioinformatics Workflow



"Find a balance between obtaining true polymorphism and introducing sequencing error"

Bioinformatics Workflow



"Find a balance between obtaining true polymorphism and introducing sequencing error"

Bioinformatics Workflow

ddRAD sequence data

↓

process_radtags

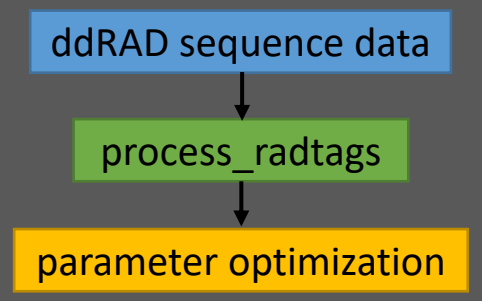
↓

parameter optimization

m = 3
M = 1-9 **denovo_map.pl -> populations -r 0.80**
n = 1-9
n = M

"a locus must be found in 80% of individuals of a single population to be processed"

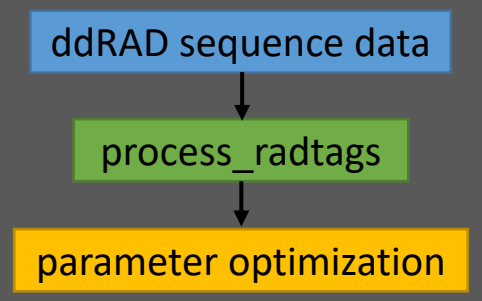
Bioinformatics Workflow



- Number of r80 polymorphic loci
- Number of new polymorphic found for each iteration of M

"Find a balance between obtaining true polymorphism and introducing sequencing error"

Bioinformatics Workflow



- Number of r80 polymorphic loci
- Number of new polymorphic found for each iteration of M

"Find a balance between obtaining true polymorphism and introducing sequencing error"

14 hours with a population map including 14 individuals

01_denovo_map_test.parameters

```bash
#!/bin/bash

#SBATCH -J denovo_map_test.parameters
#SBATCH --mail-user=YOUREMAIL@gmail.com
#SBATCH --mail-type=BEGIN,END,FAIL,TIME_LIMIT
#SBATCH --output=/work/%u/%x-%j.out
#SBATCH --error=/work/%u/%x-%j.err
#SBATCH --cpus-per-task=20
#SBATCH --mem-per-cpu=8G
#SBATCH -t 48:00:00

# Set the requested number of cores to the number of Threads your app should use
export OMP_NUM_THREADS=${SLURM_CPUS_PER_TASK:-1}

# Paths and filenames for this analysis

M_values="1 2 3 4 5 6 7 8 9"

WORK_DIR="/work/$USER/ddRAD-seq_workshop"
popmap="$WORK_DIR/data/Exercise_3/popmaps/test.popmap.txt"
OUT_DIR="$WORK_DIR/outputs/Exercise_3/test.denovo"

mkdir "$OUT_DIR"

# Create subdirectories
cd "$OUT_DIR" || exit

for M in $M_values
do
    mkdir stacks.M"$M"
done

## Load modules and activate software

module purge
module load Anaconda3
source activate /gpfs0/global/apps/stacks_2.61

# denovo_map.pl - it will execute the Stacks pipeline by running each of the Stacks components individually: ustacks, cstacks, sstacks, tsv2bam, gstacks and populations.
# We are doing this to select the parameters M (ustacks) and n (cstacks) which optimal value depends on the amount of genetic diversity within the species and with the quality of the raw data as well.
# Therefore this has to be done with every species separately, with only a subset of samples from all the populations. This subset is written in the test.popmap files and therefore Stacks will only
# run the analyses over those samples specified. We will vary M and n (M=n) from 1 to 9, and set m = 3.

# -samples = file path to the samples (samples will be read from population map)
# --popmap = file path to the population map (<sample name><TAB><population>)
# -o = file path to write the pipeline output files
# -X = additional options for specific pipeline components, e.g. -X "populations: --min-maf 0.05". We will run populations separately afterwards
# -M = number of mismatches allowed between stacks within individuals (for ustacks)
# -n =number of mismatches allowed between stacks between individuals (for cstacks)
# -m = Minimum depth of coverage required to create a stack (default 3)
# --paired = after assembling RAD loci, assemble contigs for each locus from paired-end reads
# --rm-pcr-duplicates = remove all but one set of read pairs of the same sample that have the same insert length
# -r = minimum percentage of individuals in a population required to process a locus for that population (for populations; default: 0)
# -T = the number of threads/CPUs to use (default: 1)

# Run denovo_map on the subset of samples told by the popmap

for M in $M_values
do
    out_dir="$OUT_DIR/stacks.M$M"
    reads_dir="$WORK_DIR/data/Exercise_3/demultiplexed_data/HC"
    log_file="$out_dir"/denovo_map.oe
    denovo_map.pl --samples "$reads_dir" --popmap "$popmap" -o "$out_dir" -T "$SLURM_CPUS_PER_TASK" -M "$M" -n "$M" -m 3 --paired &> "$log_file"
done

# Run populations with '-r 0.80' (loci present in 80% of samples)

for M in $M_values
do
    stacks_dir=stacks.M"$M"
    out_dir="$stacks_dir"/populations.r80
    mkdir "$out_dir"
    log_file="$out_dir"/populations.oe
    populations -P "$stacks_dir" -O "$out_dir" -t "$SLURM_CPUS_PER_TASK" -r 0.80 &> "$log_file"
done
```

01_denovo_map_test.parameters

```bash
#!/bin/bash

#SBATCH -J denovo_map_test.parameters
#SBATCH --mail-user=YOUREMAIL@gmail.com
#SBATCH --mail-type=BEGIN,END,FAIL,TIME_LIMIT
#SBATCH --output=/work/%u/%x-%j.out
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#SBATCH --cpus-per-task=20
#SBATCH --mem-per-cpu=8G
#SBATCH -t 48:00:00

# Set the requested number of cores to the number of Threads your app should use
export OMP_NUM_THREADS=${SLURM_CPUS_PER_TASK:-1}

# Paths and filenames for this analysis

M_values="1 2 3 4 5 6 7 8 9"

WORK_DIR="/work/$USER/ddRAD-seq_workshop"
popmap="$WORK_DIR/data/Exercise_3/popmaps/test.popmap.txt"
OUT_DIR="$WORK_DIR/outputs/Exercise_3/test.denovo"

mkdir "$OUT_DIR"

# Create subdirectories
cd "$OUT_DIR" || exit

for M in $M_values
do
    mkdir stacks.M"$M"
done
```

01_denovo_map_test.parameters

```
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# --rm-pcr-duplicates = remove all but one set of read pairs of the same sample that have the same insert length
# -r = minimum percentage of individuals in a population required to process a locus for that population
#(for populations; default: 0)
# -T = the number of threads/CPUs to use (default: 1)

# Run denovo_map on the subset of samples told by the popmap

for M in $M_values
do
    out_dir="$OUT_DIR/stacks.M$M"
    reads_dir="$WORK_DIR/data/Exercise_3/demultiplexed_data/HC"
    log_file="$out_dir"/denovo_map.oe
    denovo_map.pl --samples "$reads_dir" --popmap "$popmap" -o "$out_dir"\
    -T "$SLURM_CPUS_PER_TASK" -M "$M" -n "$M" -m 3 --paired &> "$log_file"
done

# Run populations with '-r 0.80' (loci present in 80% of samples)

for M in $M_values
do
    stacks_dir=stacks.M"$M"
    out_dir="$stacks_dir"/populations.r80
    mkdir "$out_dir"
    log_file="$out_dir"/populations.oe
    populations -P "$stacks_dir" -O "$out_dir" -t "$SLURM_CPUS_PER_TASK" -r 0.80 &> "$log_file"
done
```

stacks.M1	File folder	15-Jun-22 16:19:34
stacks.M2	File folder	15-Jun-22 16:19:08
stacks.M3	File folder	15-Jun-22 16:19:13
stacks.M4	File folder	15-Jun-22 16:19:03
stacks.M5	File folder	15-Jun-22 16:19:24
stacks.M6	File folder	15-Jun-22 16:18:59
stacks.M7	File folder	15-Jun-22 16:19:39
stacks.M8	File folder	15-Jun-22 16:19:29
stacks.M9	File folder	15-Jun-22 16:19:18

- populations.r80
- catalog.alleles.tsv
- catalog.calls
- catalog.fa.gz
- catalog.snps.tsv
- catalog.tags.tsv
- denovo_map.log
- denovo_map.oe
- gstacks.log
- gstacks.log.distribs
- HC1_LM5.alleles.tsv
- HC1_LM5.matches.bam
- HC1_LM5.matches.tsv
- HC1_LM5.snps.tsv
- HC1_LM5.tags.tsv
- HC1_LM9.alleles.tsv
- HC1_LM9.matches.bam
- HC1_LM9.matches.tsv
- HC1_LM9.snps.tsv
- HC1_LM9.tags.tsv

- populations.haplotypes.tsv
- populations.hapstats.tsv
- populations.log
- populations.log.distribs
- populations.sumstats.tsv
- populations.sumstats_summary.tsv
- tsv2bam.log

stacks.M1	File folder	15-Jun-22 16:19:34
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stacks.M9	File folder	15-Jun-22 16:19:18

- populations.r80
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- catalog.tags.tsv
- denovo_map.log
- denovo_map.oe
- gstacks.log
- gstacks.log.distribs
- HC1_LM5.alleles.tsv
- HC1_LM5.matches.bam
- HC1_LM5.matches.tsv
- HC1_LM5.snps.tsv
- HC1_LM5.tags.tsv
- HC1_LM9.alleles.tsv
- HC1_LM9.matches.bam
- HC1_LM9.matches.tsv
- HC1_LM9.snps.tsv
- HC1_LM9.tags.tsv

- populations.haplotypes.tsv
- populations.hapstats.tsv
- populations.log
- populations.log.distribs
- populations.sumstats.tsv
- populations.sumstats_summary.tsv
- tsv2bam.log

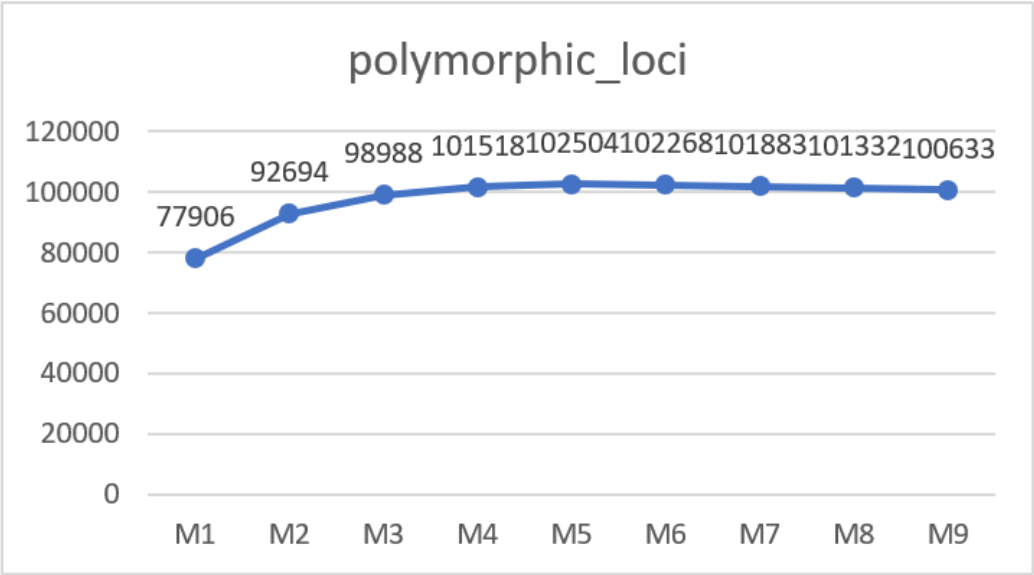
Extract this information:
Number of r80 polymorphic loci
- Number of new polymorphic found for each iteration of M

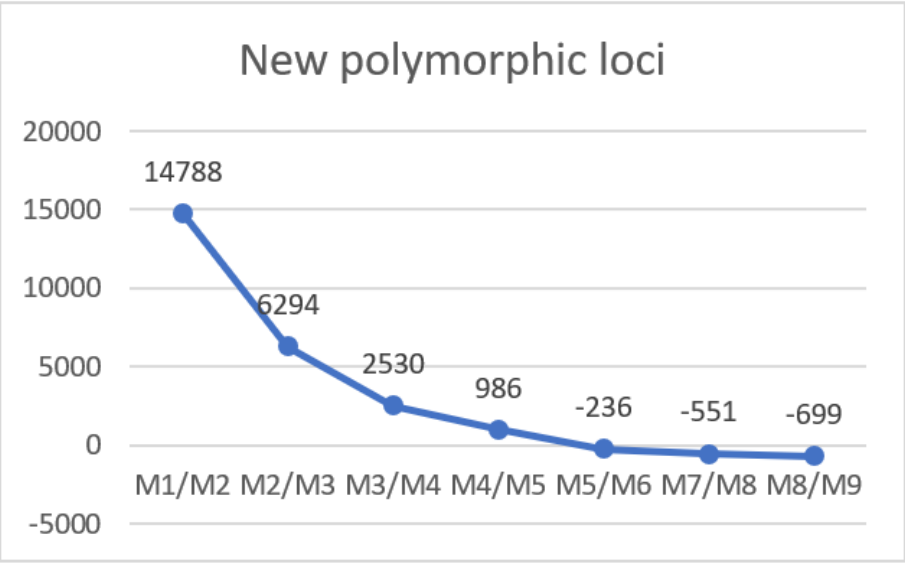
02_extract_results

```bash
#!/bin/bash

#SBATCH -J extract_results
#SBATCH --mail-user=YOUREMAIL@gmail.com
#SBATCH --mail-type=BEGIN,END,FAIL,TIME_LIMIT
#SBATCH --output=/work/%u/%x-%j.out
#SBATCH --error=/work/%u/%x-%j.err
#SBATCH --mem-per-cpu=4G
#SBATCH -t 1:00:00

# Paths and filenames for this analysis

M_values="1 2 3 4 5 6 7 8 9"

WORK_DIR="/work/$USER/ddRAD-seq_workshop/outputs/Exercise_3/test.denovo"

## Load modules and activate software

module purge
module load Anaconda3
source activate /gpfs0/global/apps/stacks_2.61

cd "$WORK_DIR" || exit
mkdir "$WORK_DIR/results"


for M in $M_values
do
stacks-dist-extract stacks.M"$M"/populations.r80/populations.log.distribs snps_per_loc_postfilters >> results/M"$M"_snp_distribution.tsv
cat stacks.M"$M"/populations.r80/populations.sumstats.tsv | grep -v "^#" | cut -f 1 | sort -n | uniq | wc -l >> results/M"$M"_r80.polymorphicLOCI.tsv
awk 'NR == 6 {print $5}' stacks.M"$M"/populations.r80/populations.sumstats_summary.tsv >> results/M"$M"_r80.polymorphicLOCI_summary.tsv
cat results/*.polymorphicLOCI.tsv >> results/all.polymorphicLOCI.FINAL.tsv
cat  results/*.polymorphicLOCI_summary.tsv > results/all.polymorphicLOCI_summary.FINAL.tsv
done
```

Bioinformatics

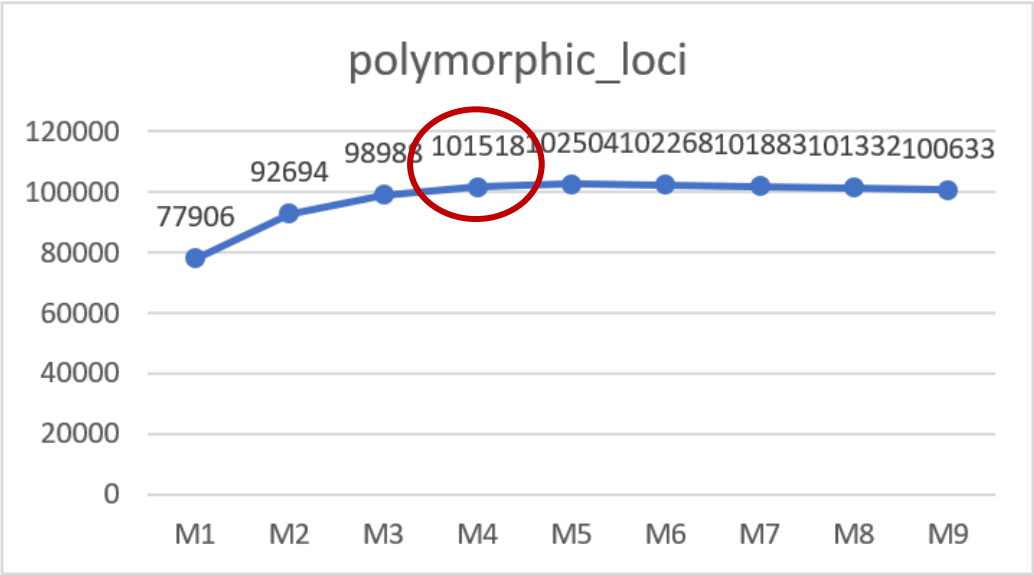
Bismarckia nobilis

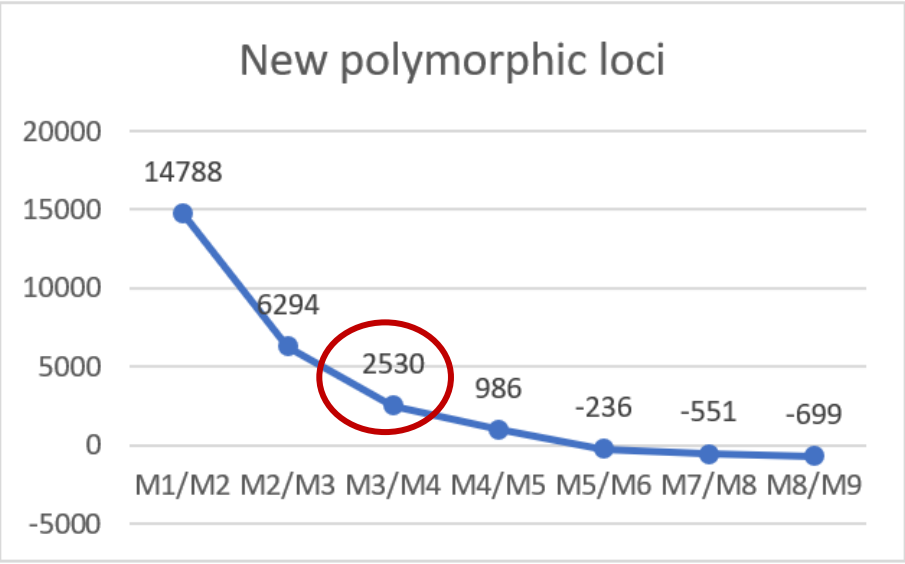




Bioinformatics

Bismarckia nobilis

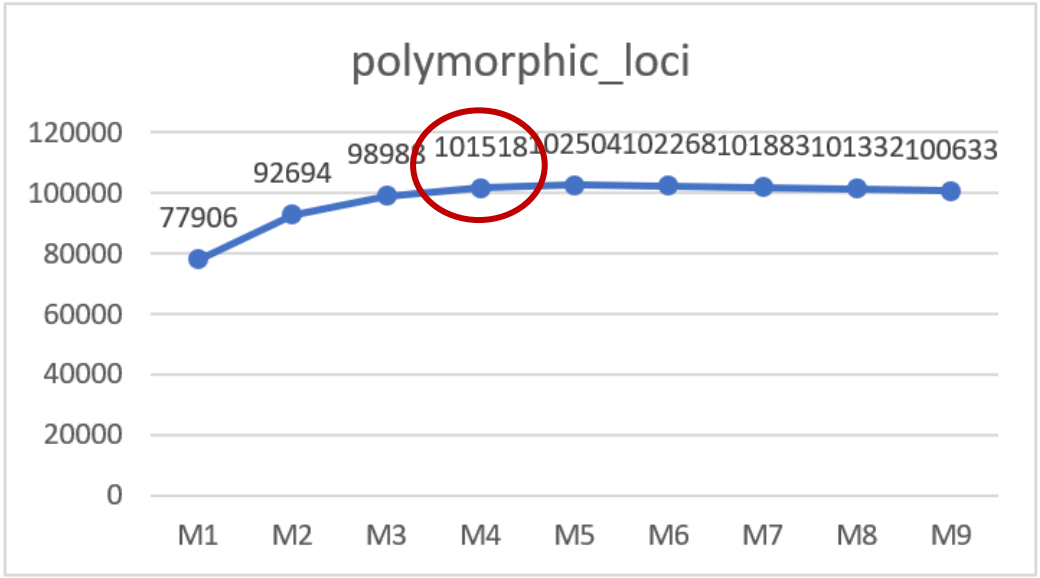


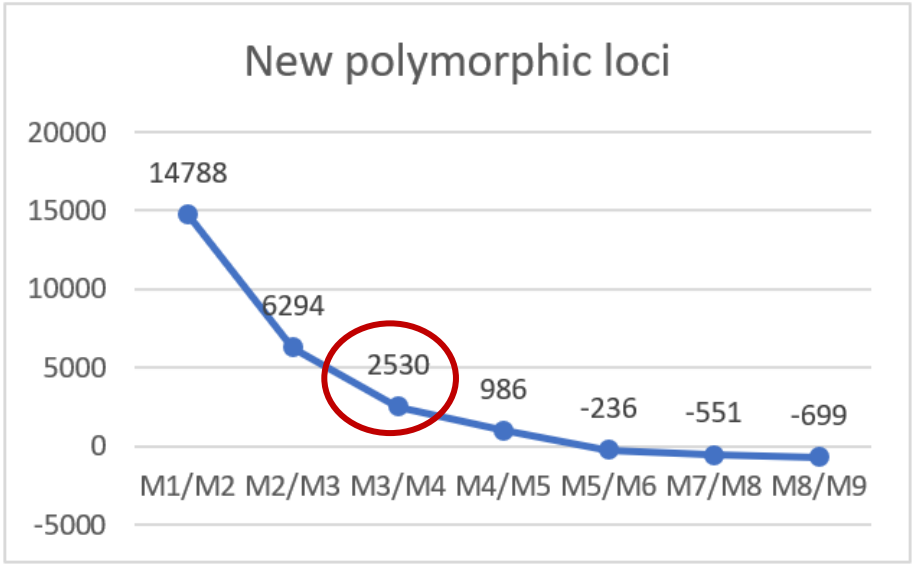


"Find a balance between obtaining true polymorphism and introducing sequencing error"

Bioinformatics

Bismarckia nobilis

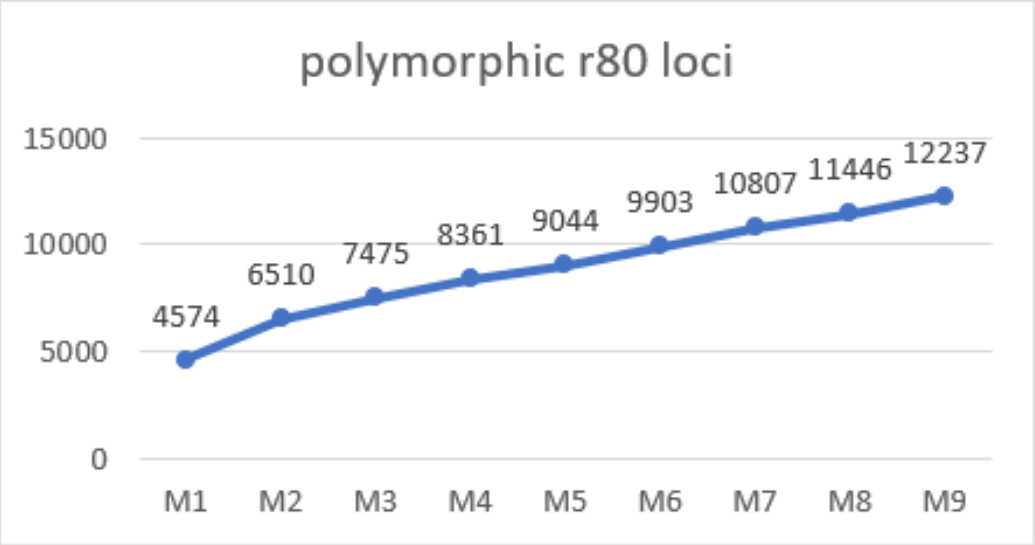


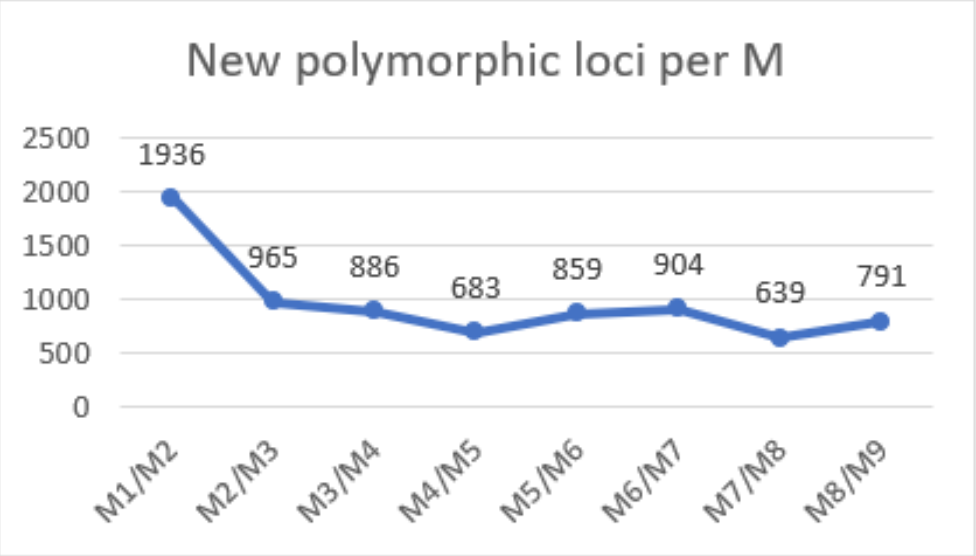


M = 4
M = n

Bioinformatics

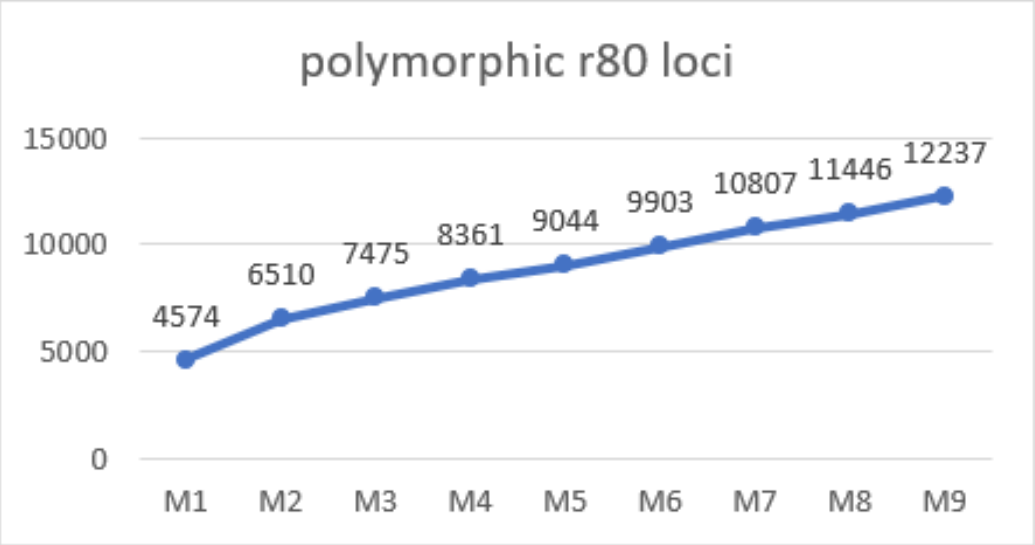
Dypsis pinnatifrons

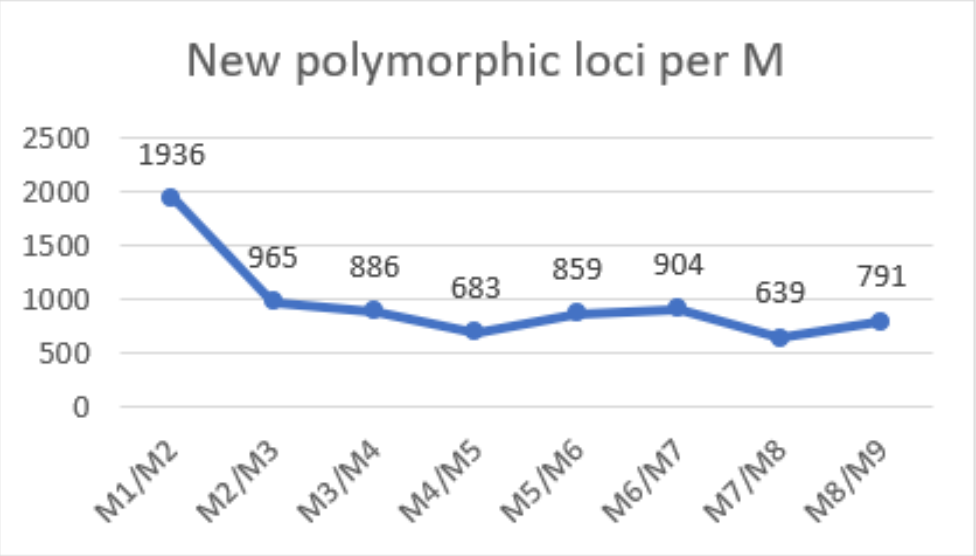




Bioinformatics

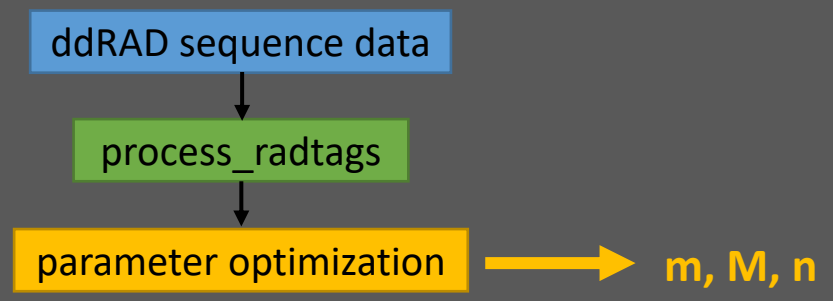
Dypsis pinnatifrons



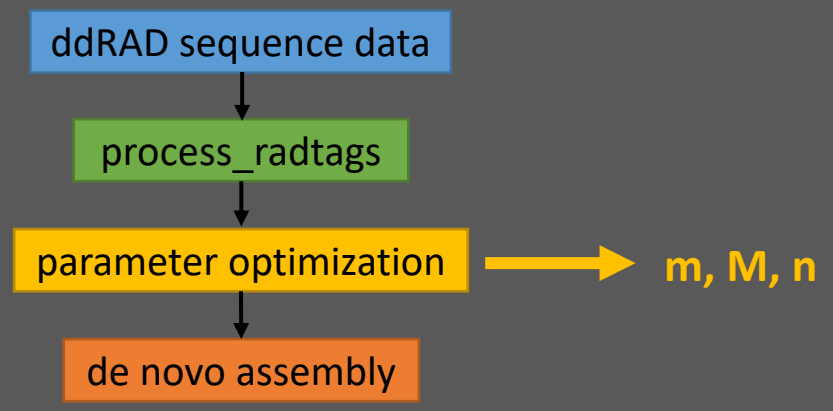


M = 5
M = n

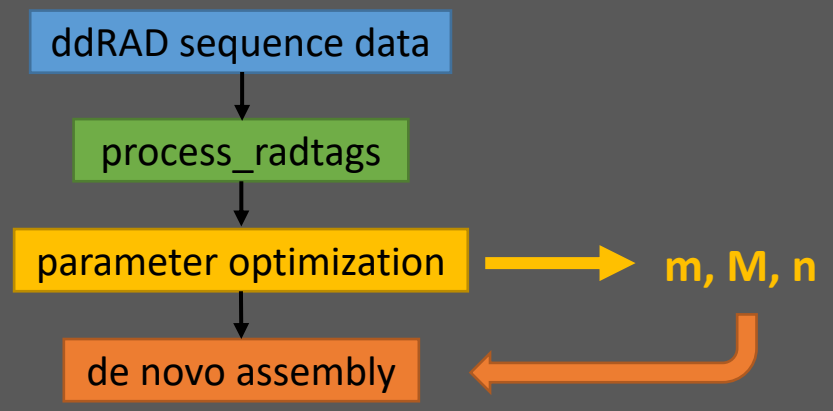
Bioinformatics Workflow



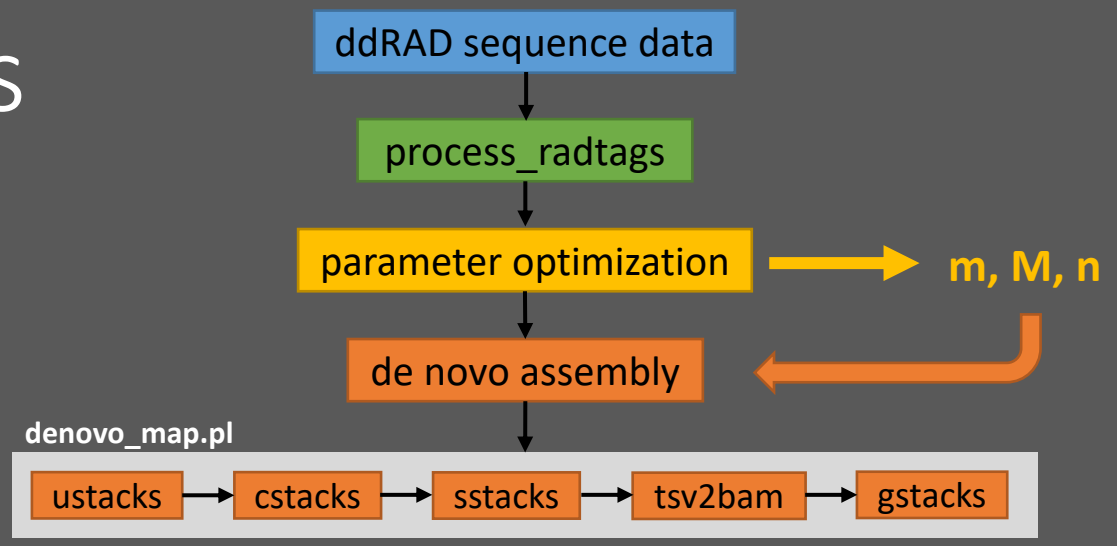
Bioinformatics Workflow



Bioinformatics Workflow



Bioinformatics Workflow



Rochette, Rivera-Colón & Catchen, 2019 - Mol. Ecology

ddRAD sequencing

ddRADseq Data

HPC cluster (EVE) - UFZ

Stacks v2.61

process_radtags — Clean and demultiplex the data

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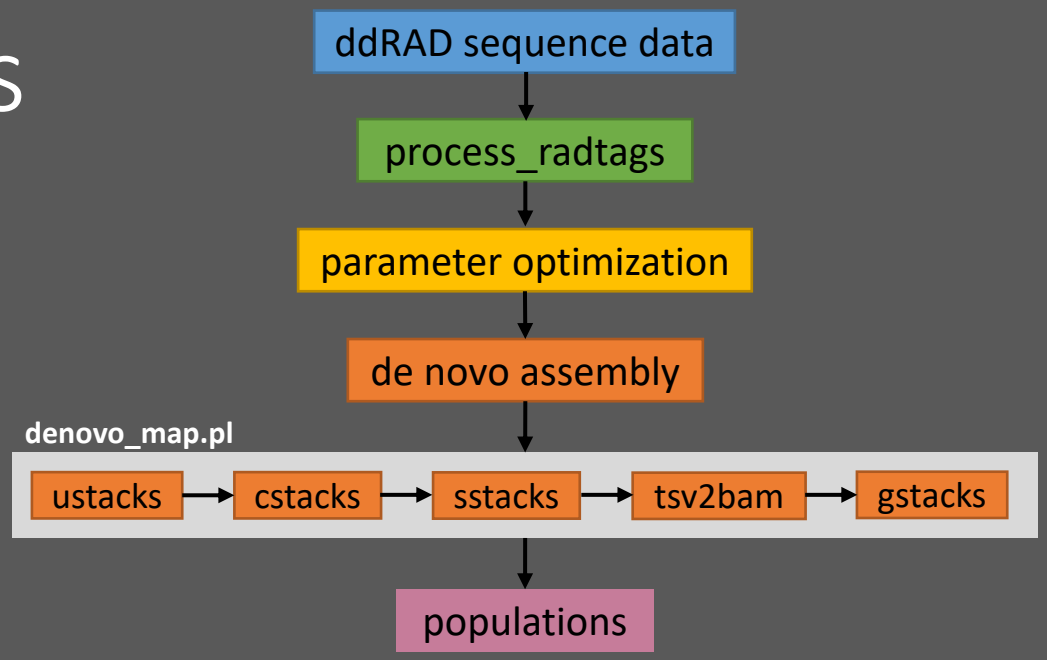
gstacks — A contig is assembled from pair-end reads and overlapped with the single-end locus. SNP calling

populations — Population level statistics and output in different formats. Possibility for further filtering

Output files for population structure analyses, phylogenetics, demographic history…

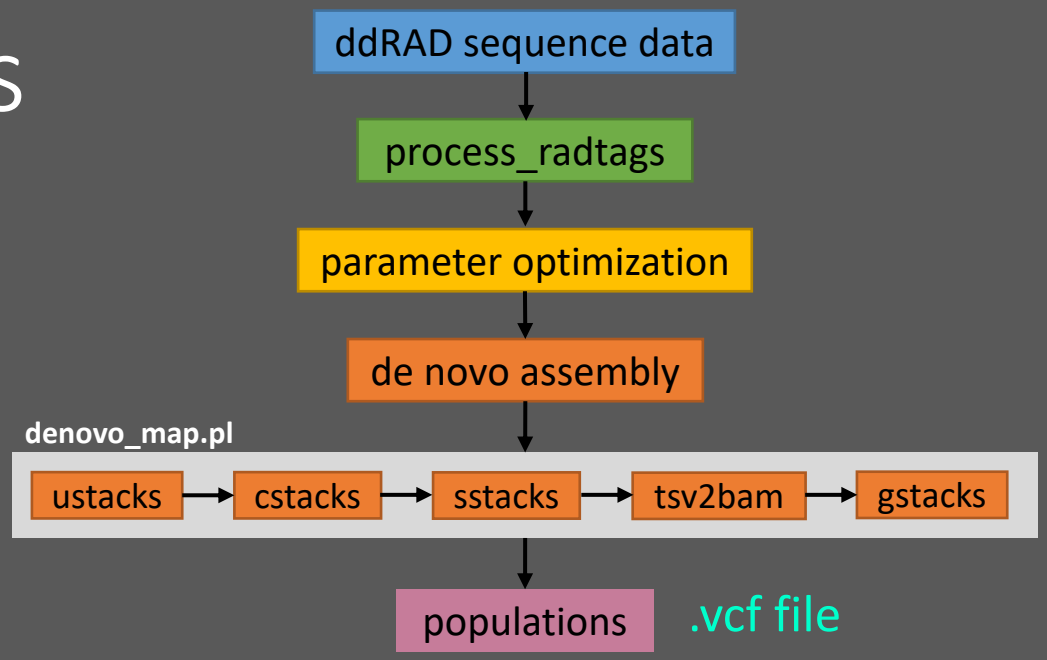
Bioinformatics Workflow



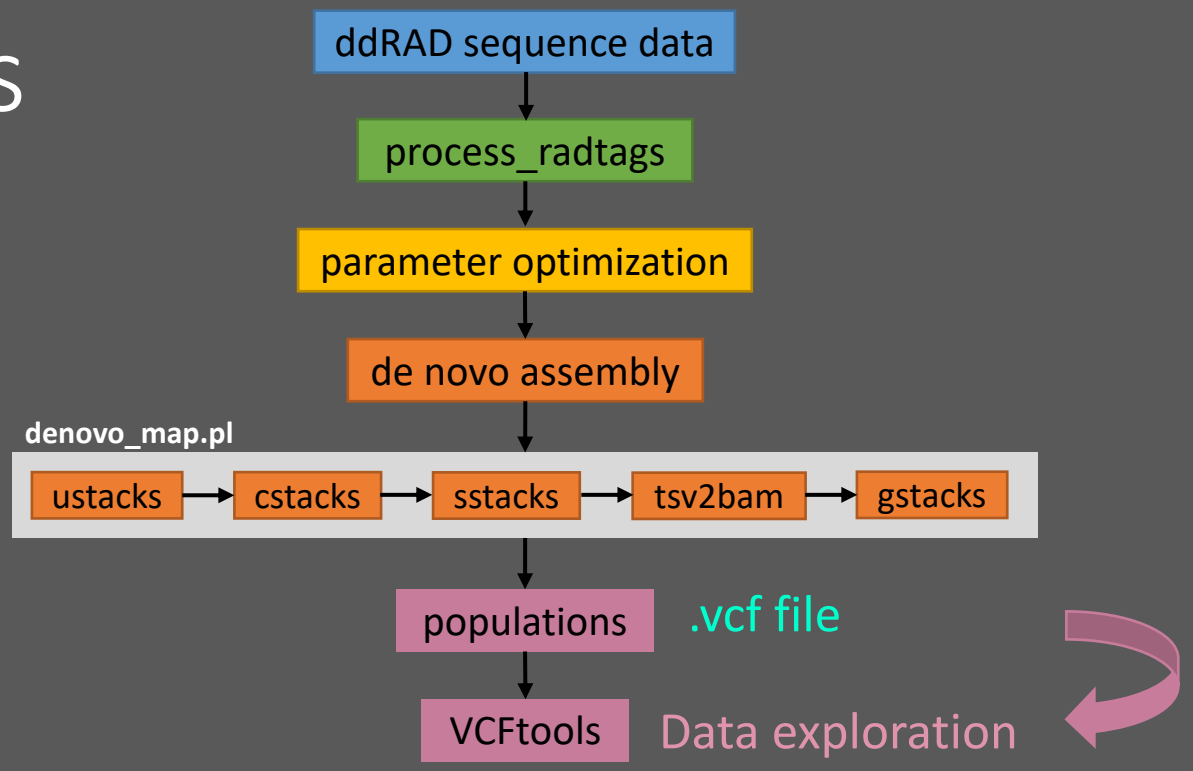
03_denovo_map_full

```
# Run denovo_map on the subset of samples told by the popmap


denovo_map.pl --samples "$reads_dir" --popmap "$popmap" -o "$out_dir" -T "$SLURM_CPUS_PER_TASK" \
-M "$M" -n "$M" -m 3 --paired -X "populations: --vcf" &> "$log_file"
```

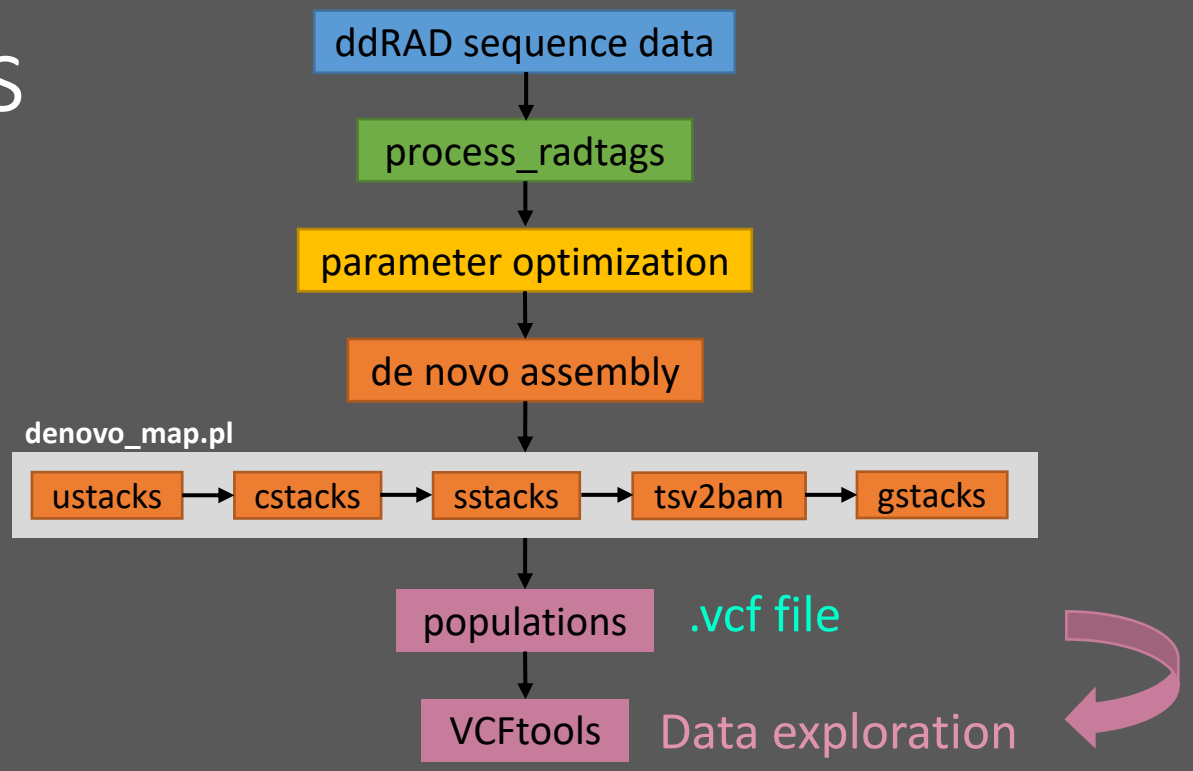
Bioinformatics Workflow



Bioinformatics Workflow



Bioinformatics Workflow



--freq2 = allele frequency

--depth = mean depth per individual

--site-mean-depth = mean depth per site

--missing-indv = proportion of missing data per individual

--missing-site = proportion of missing data per site

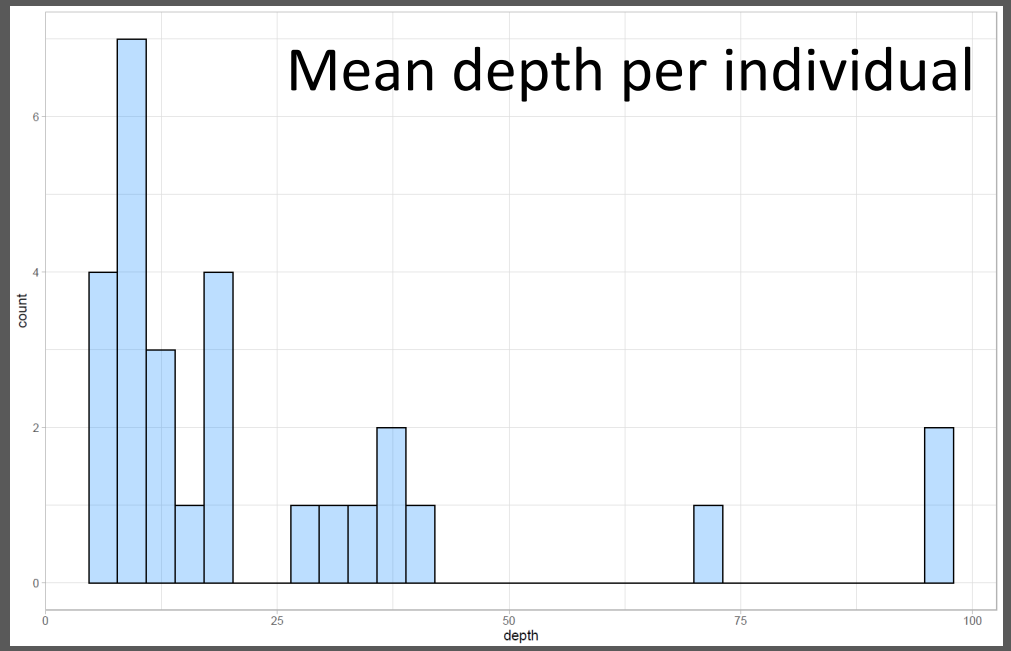
--het = heterozygosity and inbreeding coefficient per individual

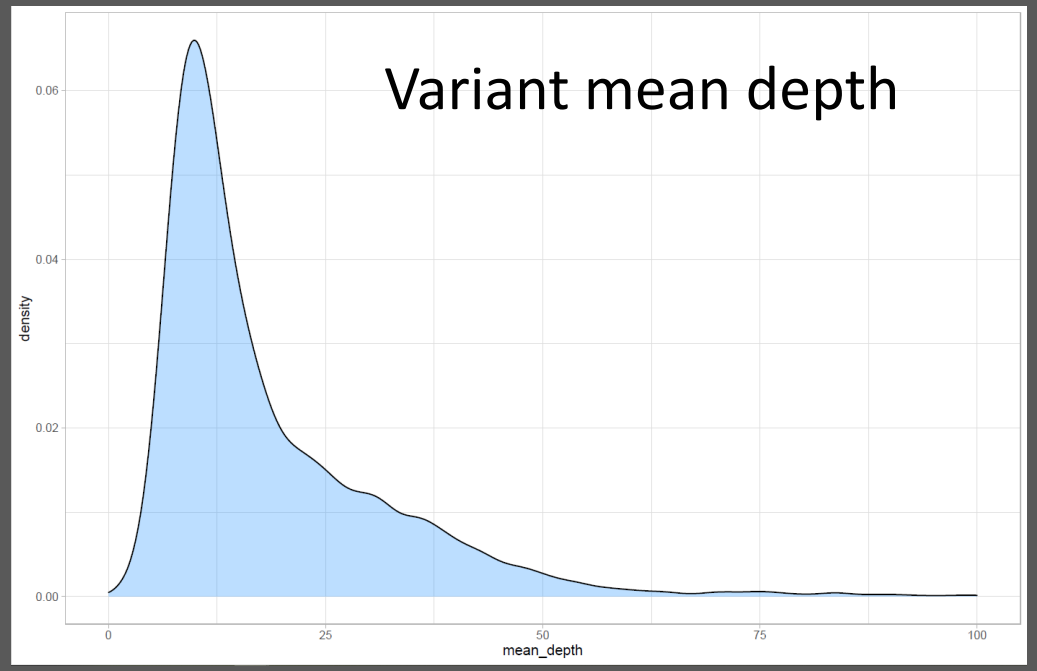
04_extract_results

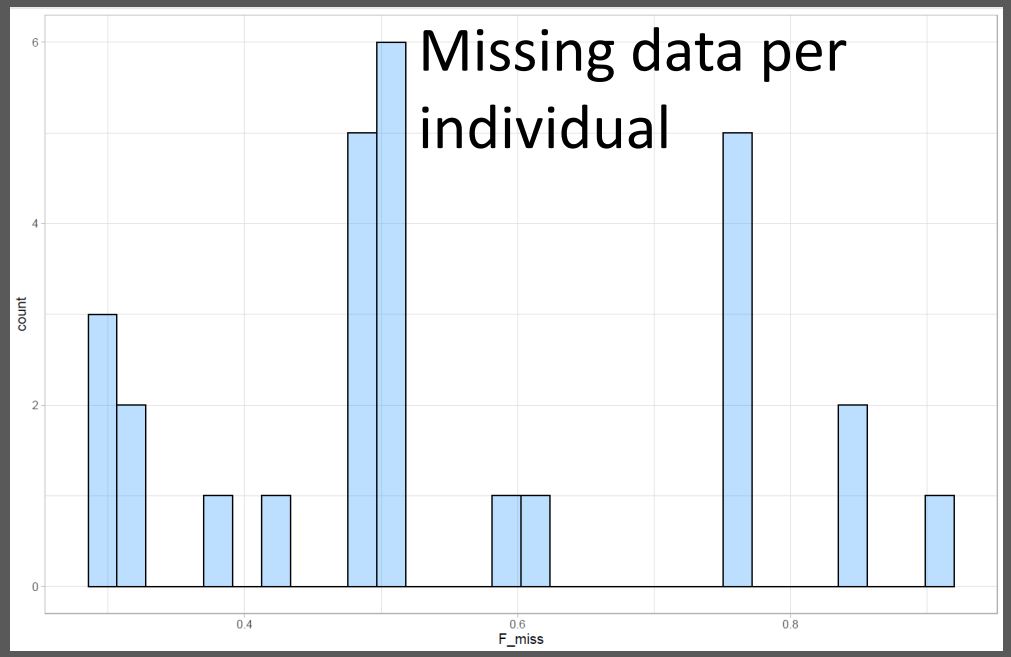
```bash
#!/bin/bash

#SBATCH -J vcftools
#SBATCH --mail-user=YOUREMAIL@gmail.com
#SBATCH --mail-type=BEGIN,END,FAIL,TIME_LIMIT
#SBATCH --output=/work/%u/%x-%j.out
#SBATCH --error=/work/%u/%x-%j.err
#SBATCH --mem-per-cpu=4G
#SBATCH -t 48:00:00

# Paths and filenames for this analysis

WORK_DIR="/work/$USER/ddRAD-seq_workshop"

out_dir="$WORK_DIR/outputs/Exercise_3/stacks.denovo/VCFtools"
cd "$WORK_DIR" || exit
mkdir "$out_dir"
vcf_dir="$WORK_DIR/outputs/Exercise_3/stacks.denovo/populations.snps.vcf"
log_file="$out_dir"/vcftools_summary.oe

## Load modules and activate software

module load foss/2019b VCFtools/0.1.16


# VCFtools - vcftools is a suite of functions for use on genetic variation data in the form of VCF and BCF files.
#The tools provided will be used mainly to summarize data, run calculations on data, filter out data, and convert data into other useful file formats.
# SYNOPSIS:
# vcftools [ --vcf FILE | --gzvcf FILE | --bcf FILE] [ --out OUTPUT PREFIX ] [ FILTERING OPTIONS ] [ OUTPUT OPTIONS ]

# Run VCFtools to calculate some basic stats from out vcf files per species

cd "$out_dir"
    vcftools --vcf "$vcf_dir" --freq2 --out "./freq2" --max-alleles 2 &> "$log_file"
    vcftools --vcf "$vcf_dir" --depth --out "./ind_depth" &> "$log_file"
    vcftools --vcf "$vcf_dir" --site-mean-depth --out "./mean_depth_site" &> "$log_file"
    vcftools --vcf "$vcf_dir" --site-quality --out "./site_quality" &> "$log_file"
    vcftools --vcf "$vcf_dir" --missing-indv --out "./missing_ind" &> "$log_file"
    vcftools --vcf "$vcf_dir" --missing-site --out "./missing_ind" &> "$log_file"
    vcftools --vcf "$vcf_dir" --het --out "./het" &> "$log_file"
```

Bioinformatics

Borassus madagascariensis
28 individuals

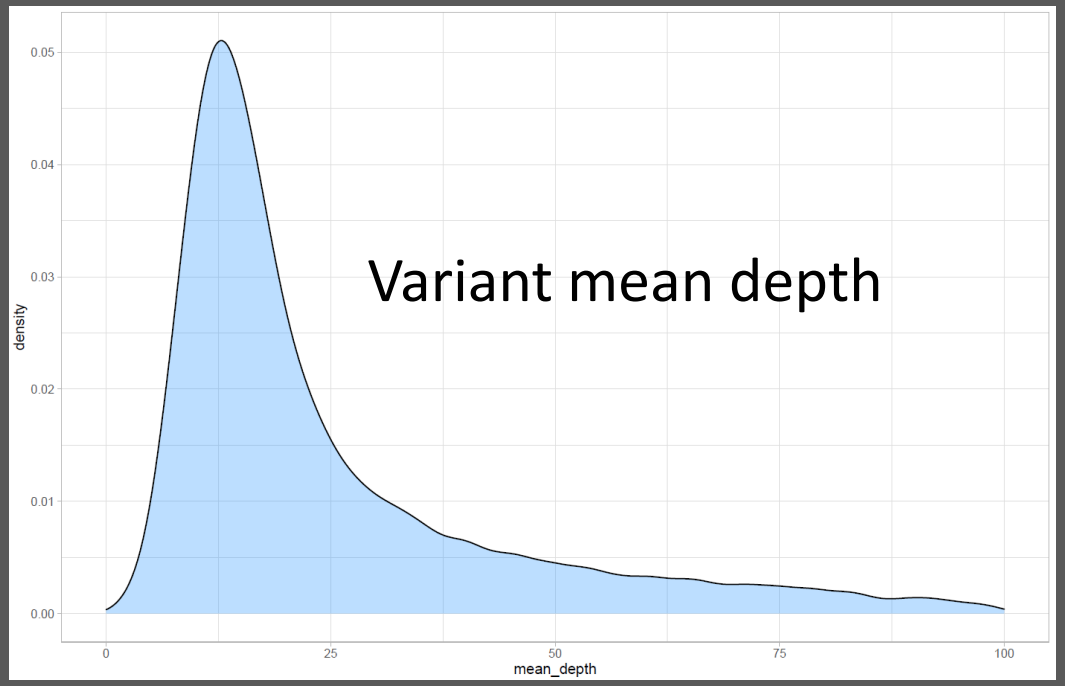


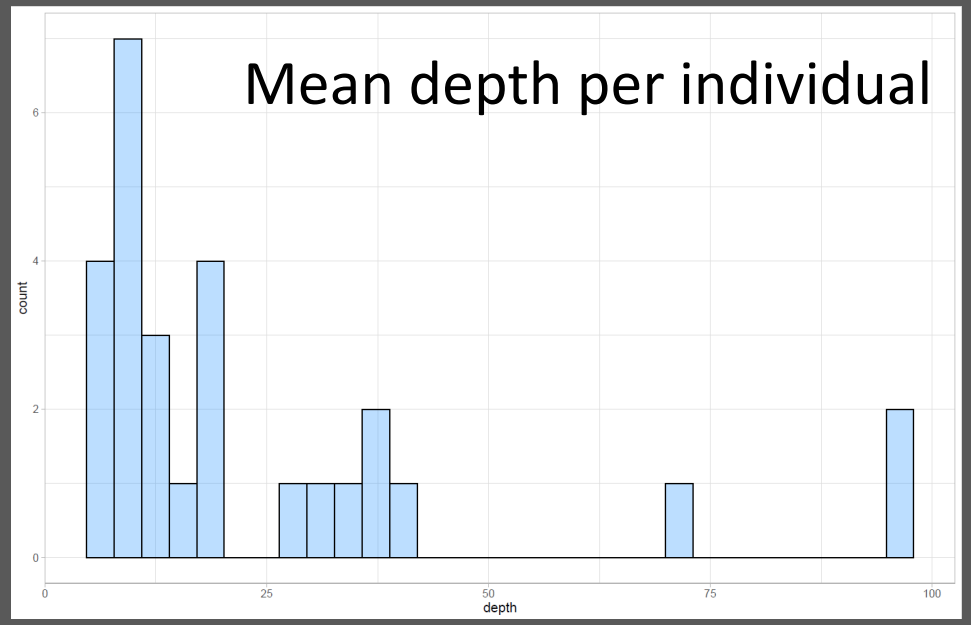


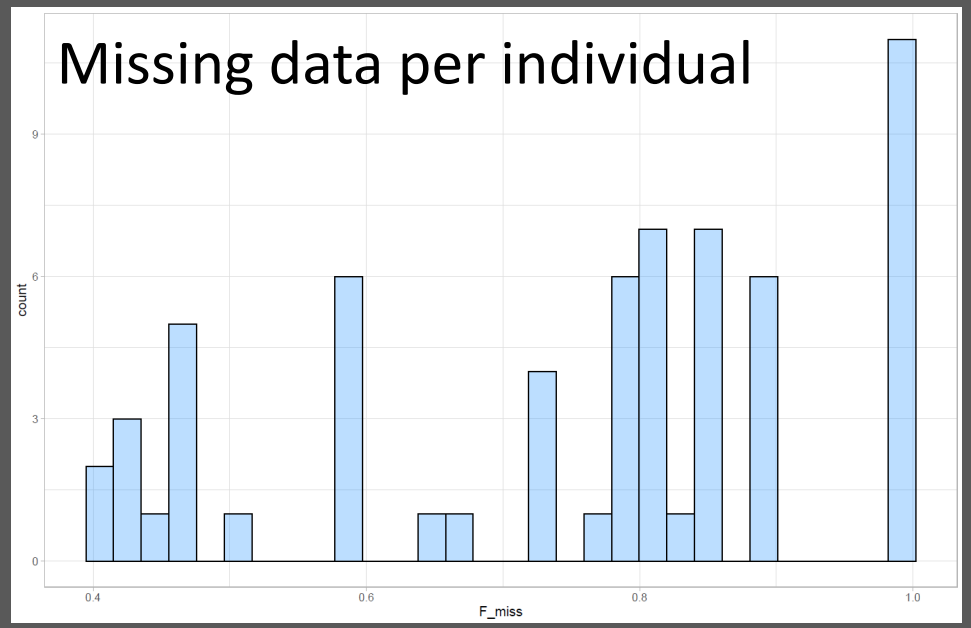


Bioinformatics

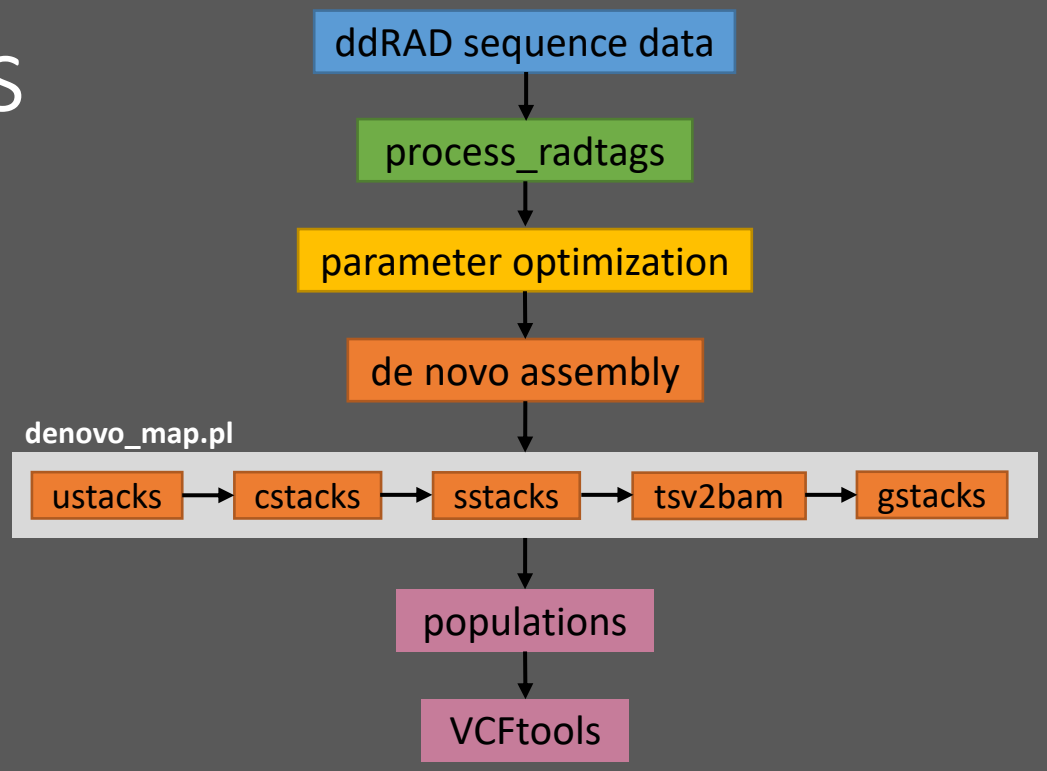
Bismarckia nobilis
63 individuals



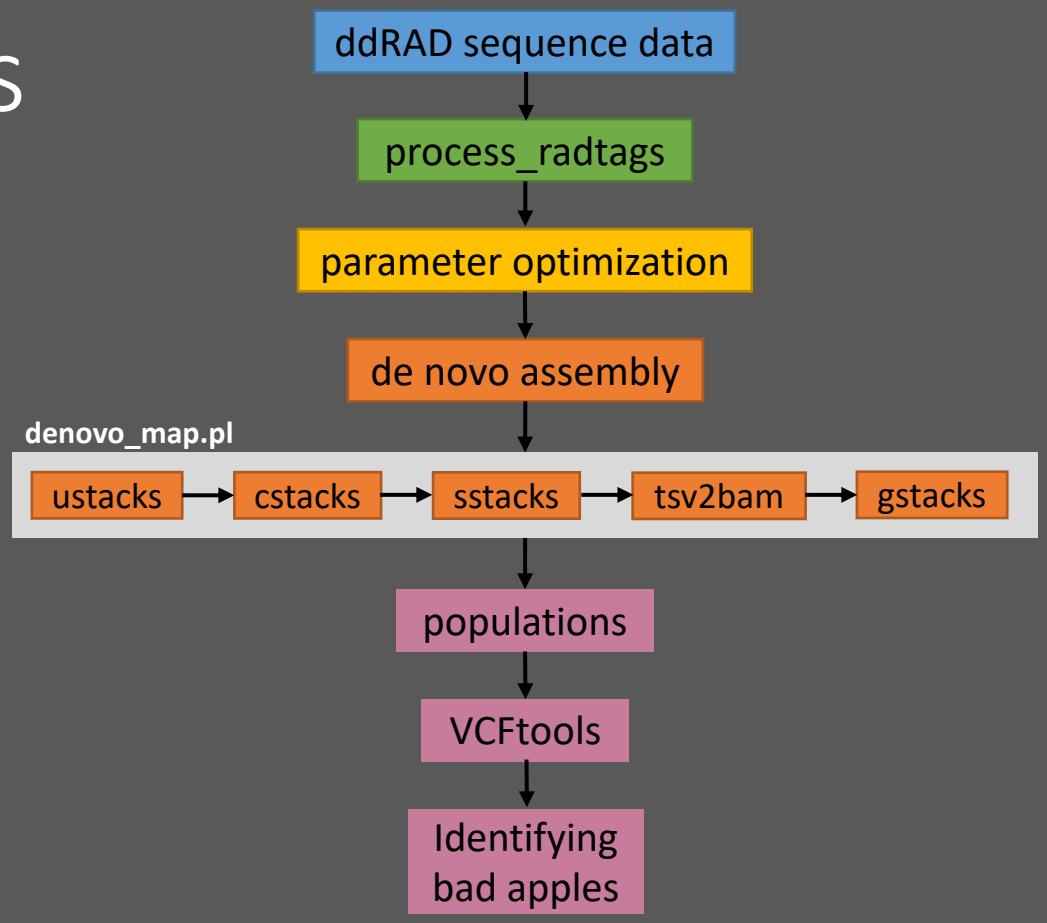




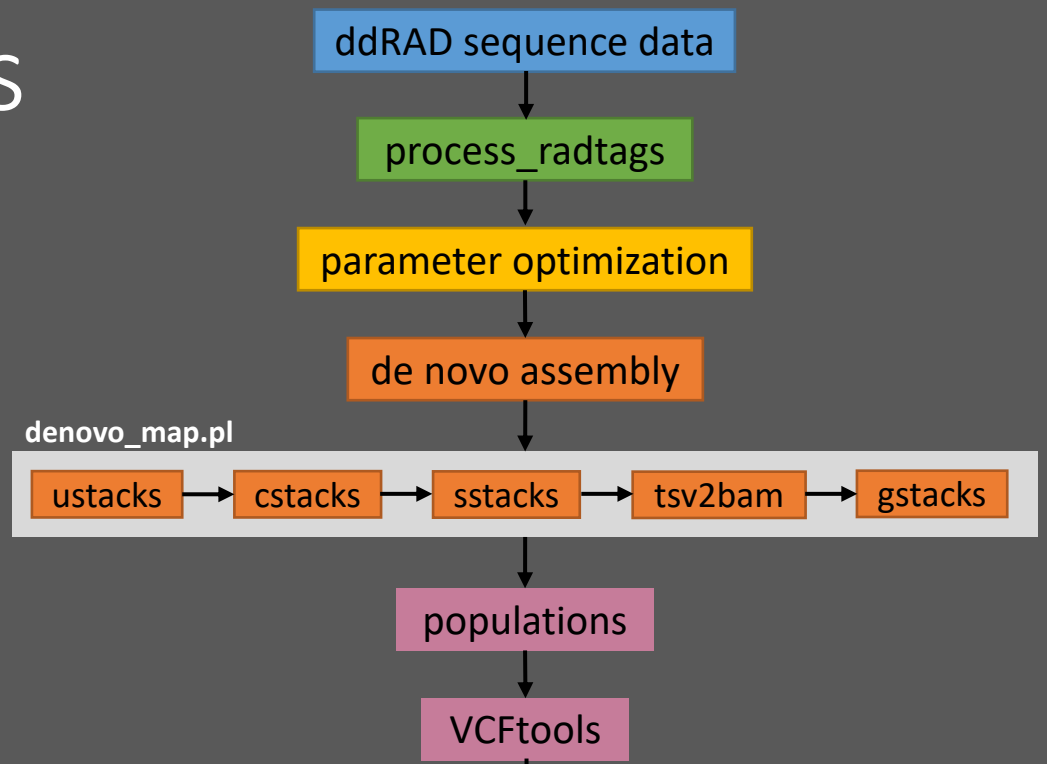
Bioinformatics Workflow



Bioinformatics Workflow



Bioinformatics Workflow



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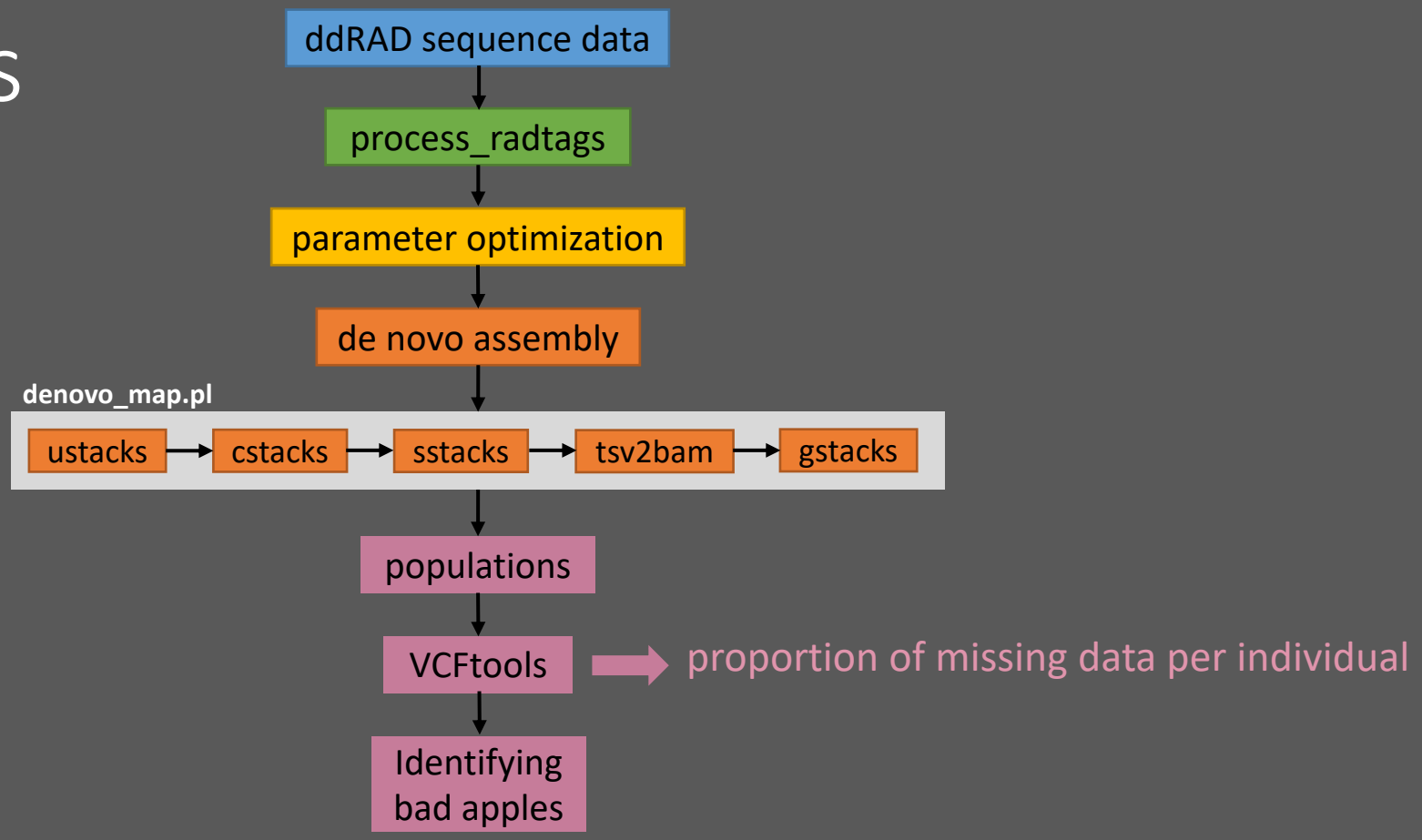
RESEARCH ARTICLE

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

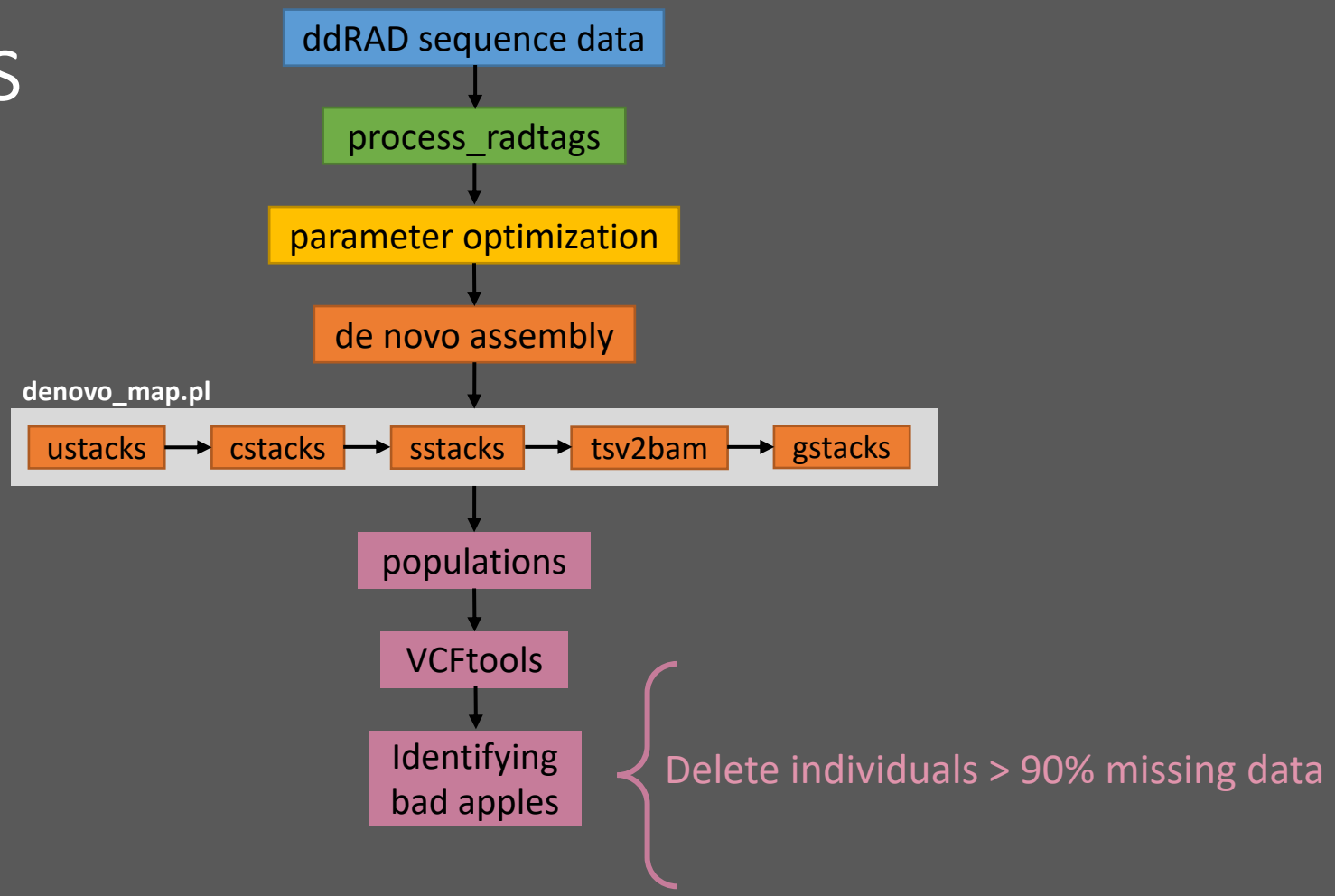
Removing the bad apples: A simple bioinformatic method to improve loci-recovery in de novo RADseq data for non-model organisms

José Cerca[1,2,3] | Marius F. Maurstad[1,4] | Nicolas C. Rochette[5,6] | Angel G. Rivera-Colón[5] | Niraj Rayamajhi[5] | Julian M. Catchen[5] | Torsten H. Struck[1]

Bioinformatics Workflow

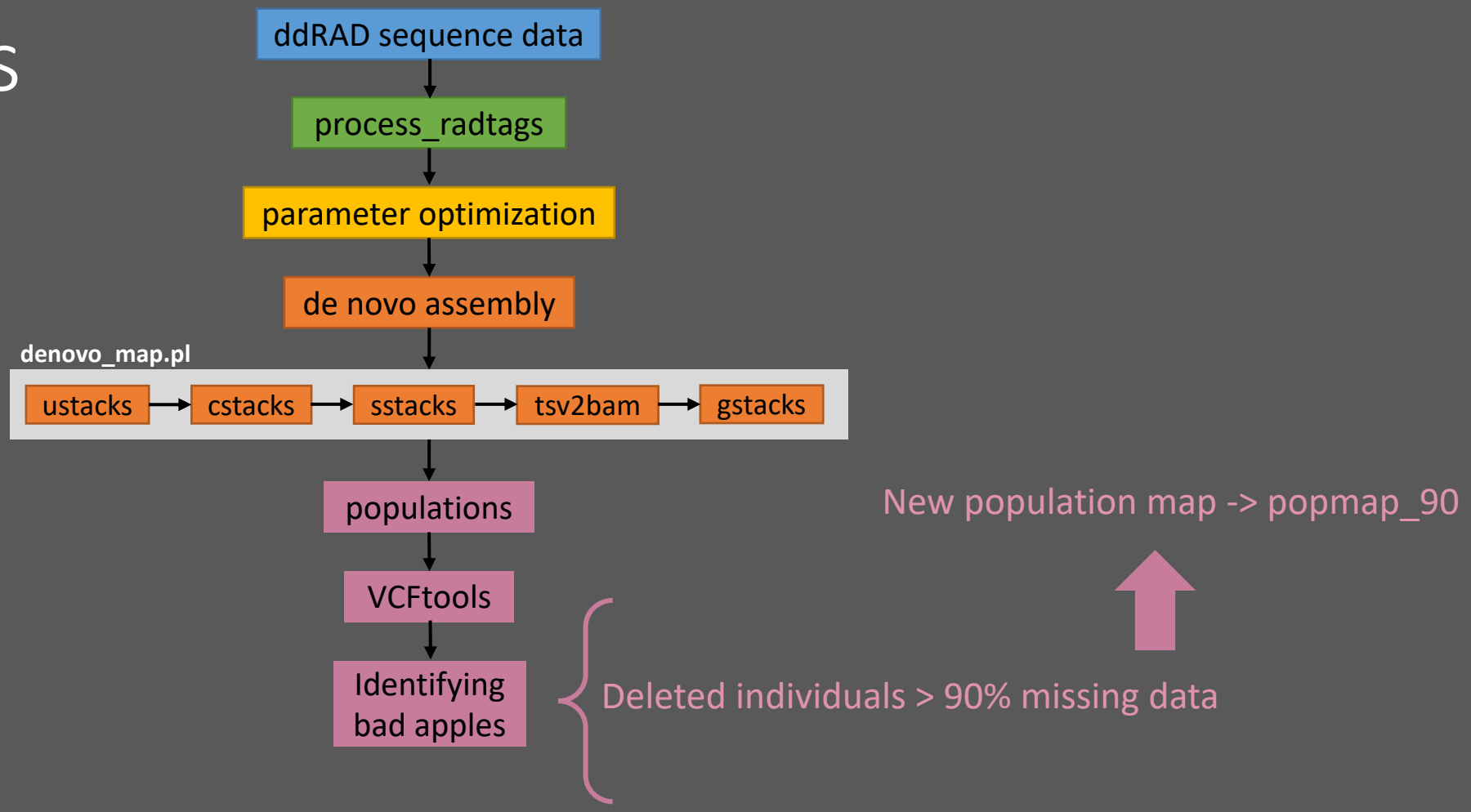


Bioinformatics Workflow



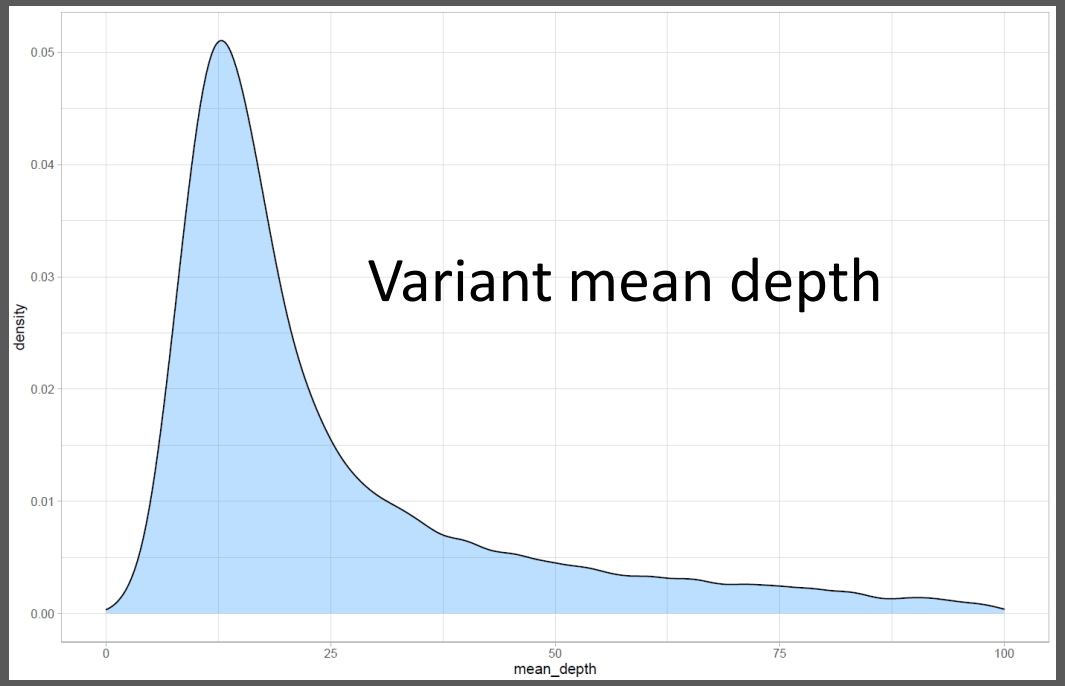
"helps recovering a higher number of loci"

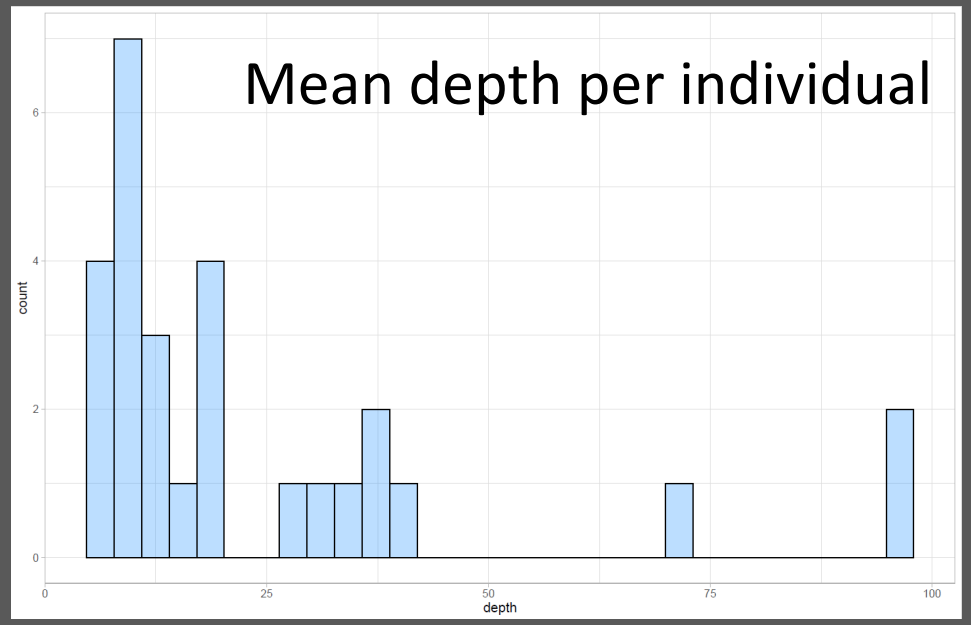
Bioinformatics Workflow

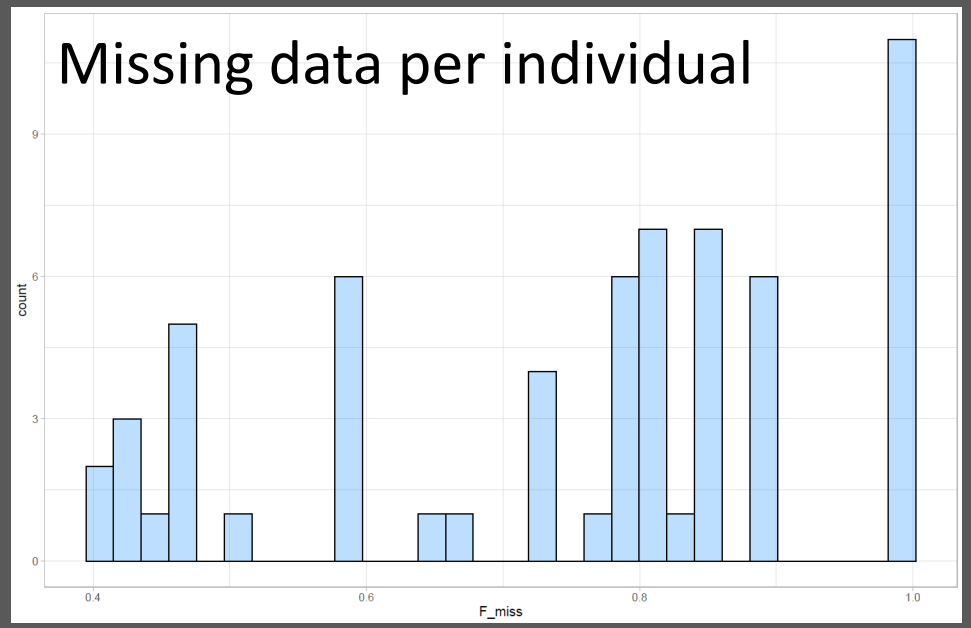


Bioinformatics

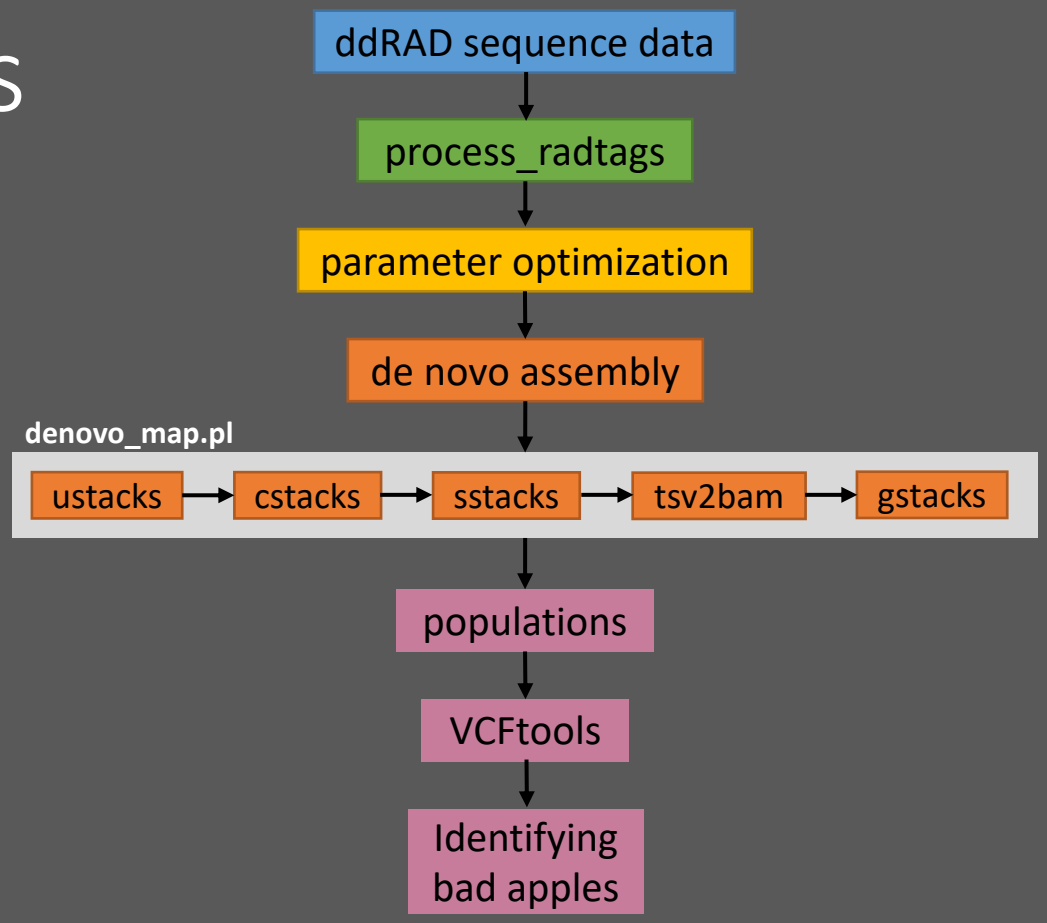
Bismarckia nobilis
63 individuals



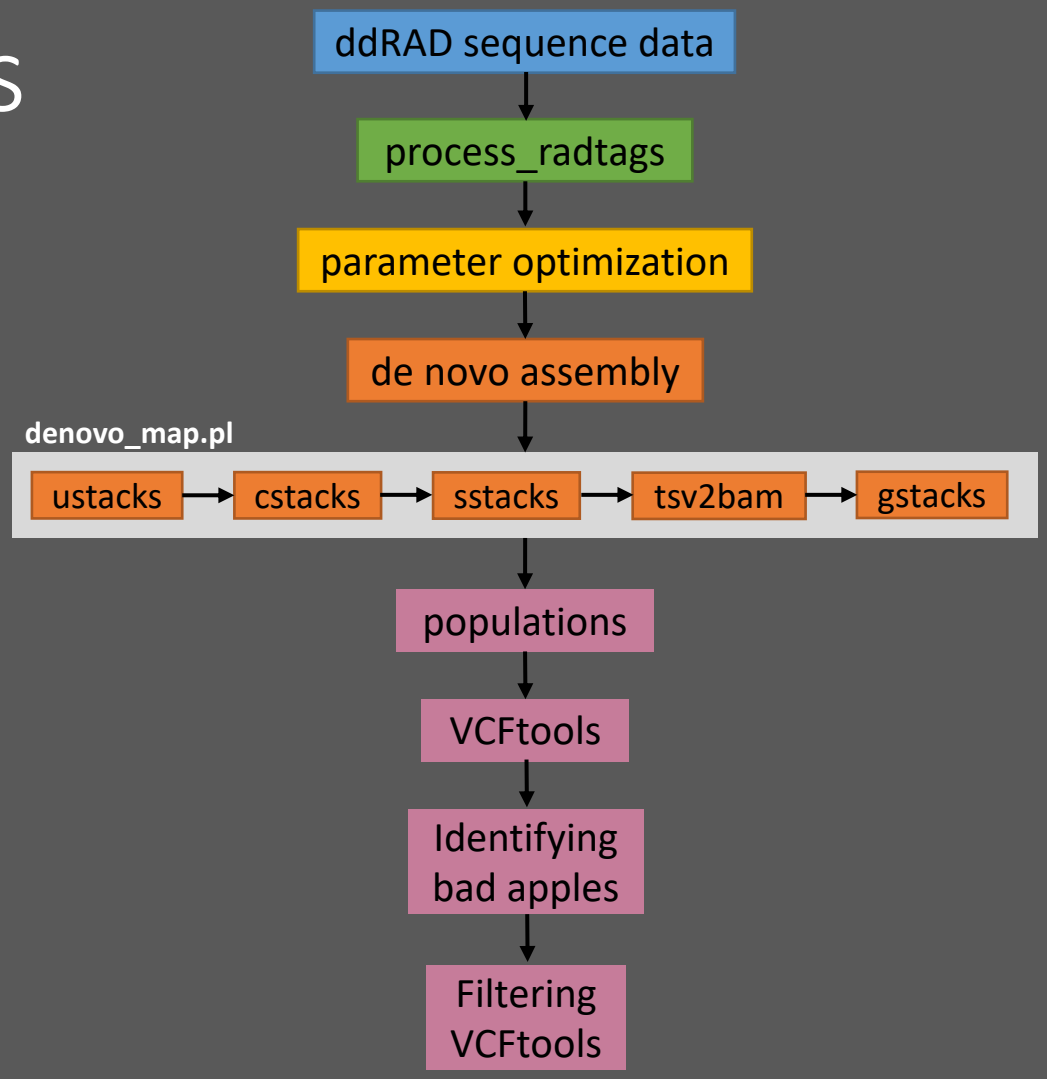




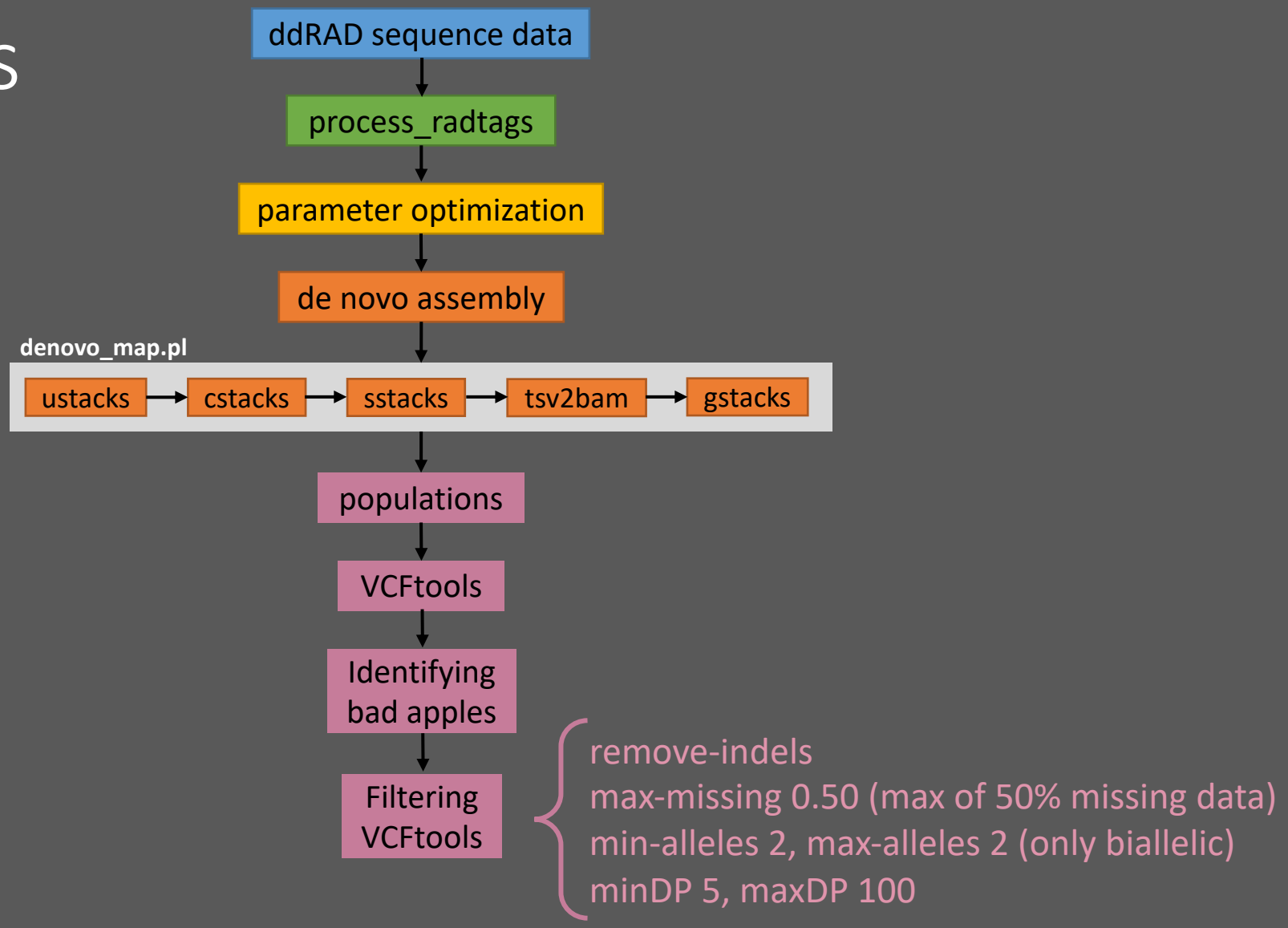
Bioinformatics Workflow



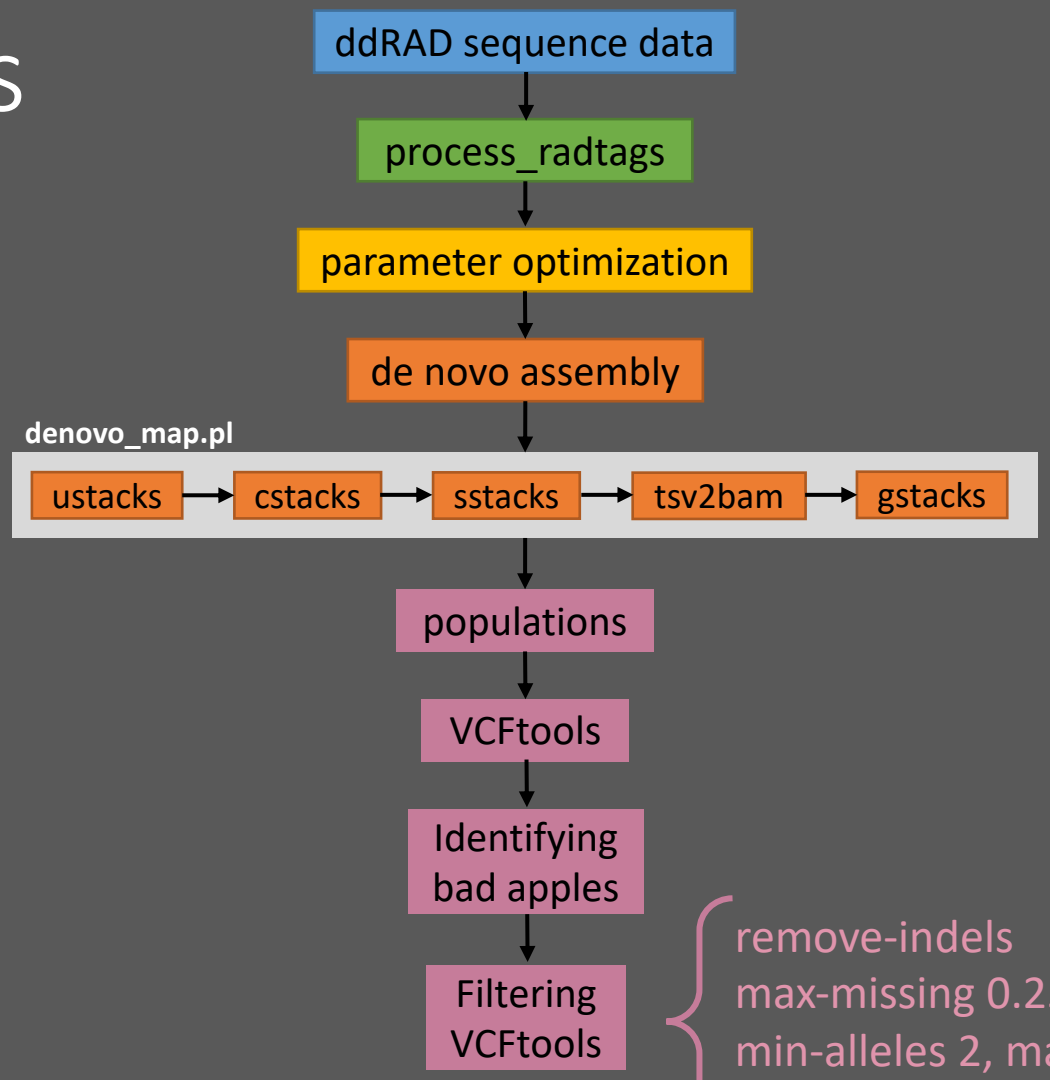
Bioinformatics Workflow



Bioinformatics Workflow



Bioinformatics Workflow



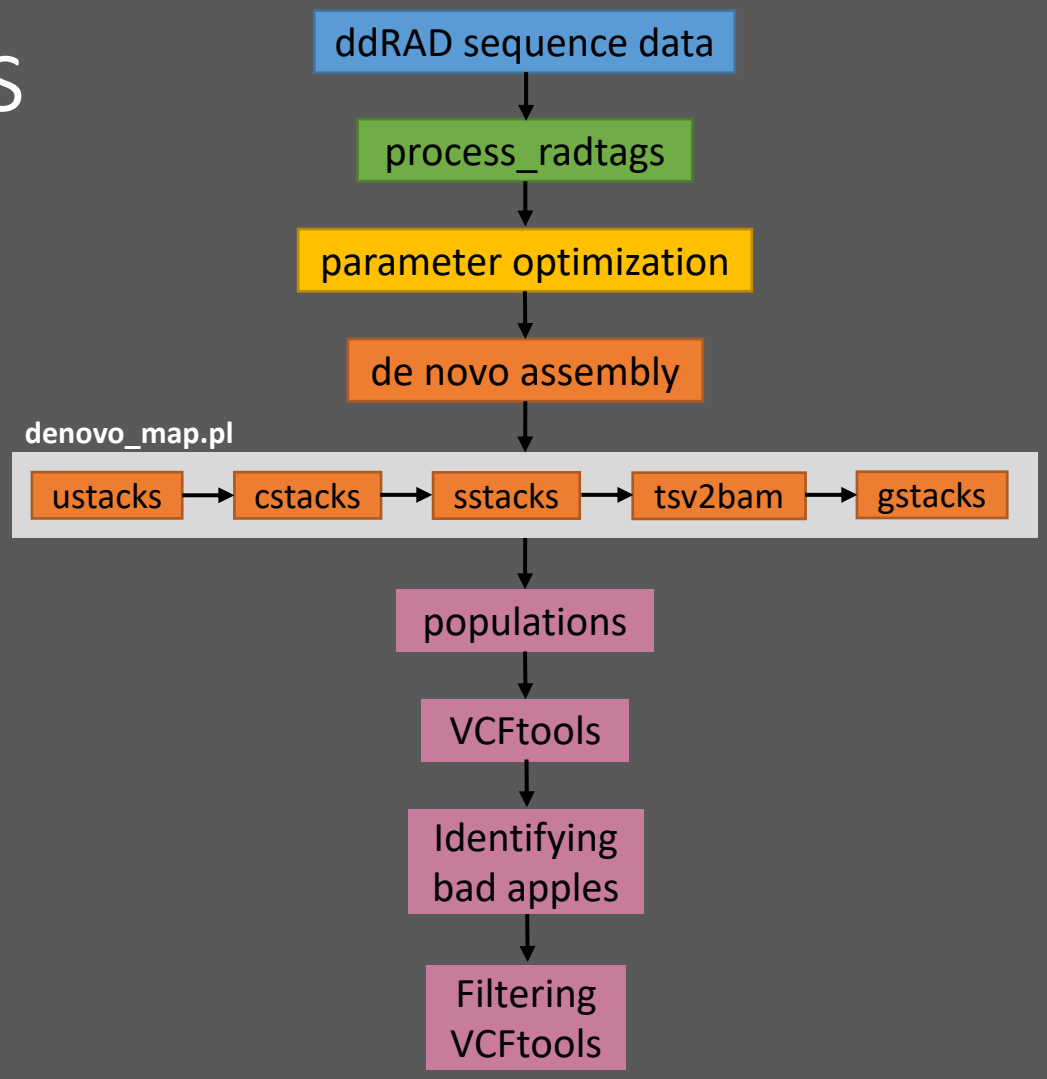
To control for false-positive loci

remove-indels
max-missing 0.25 (max of 75% missing data)
min-alleles 2, max-alleles 2 (only biallelic)
minDP 5, maxDP 100

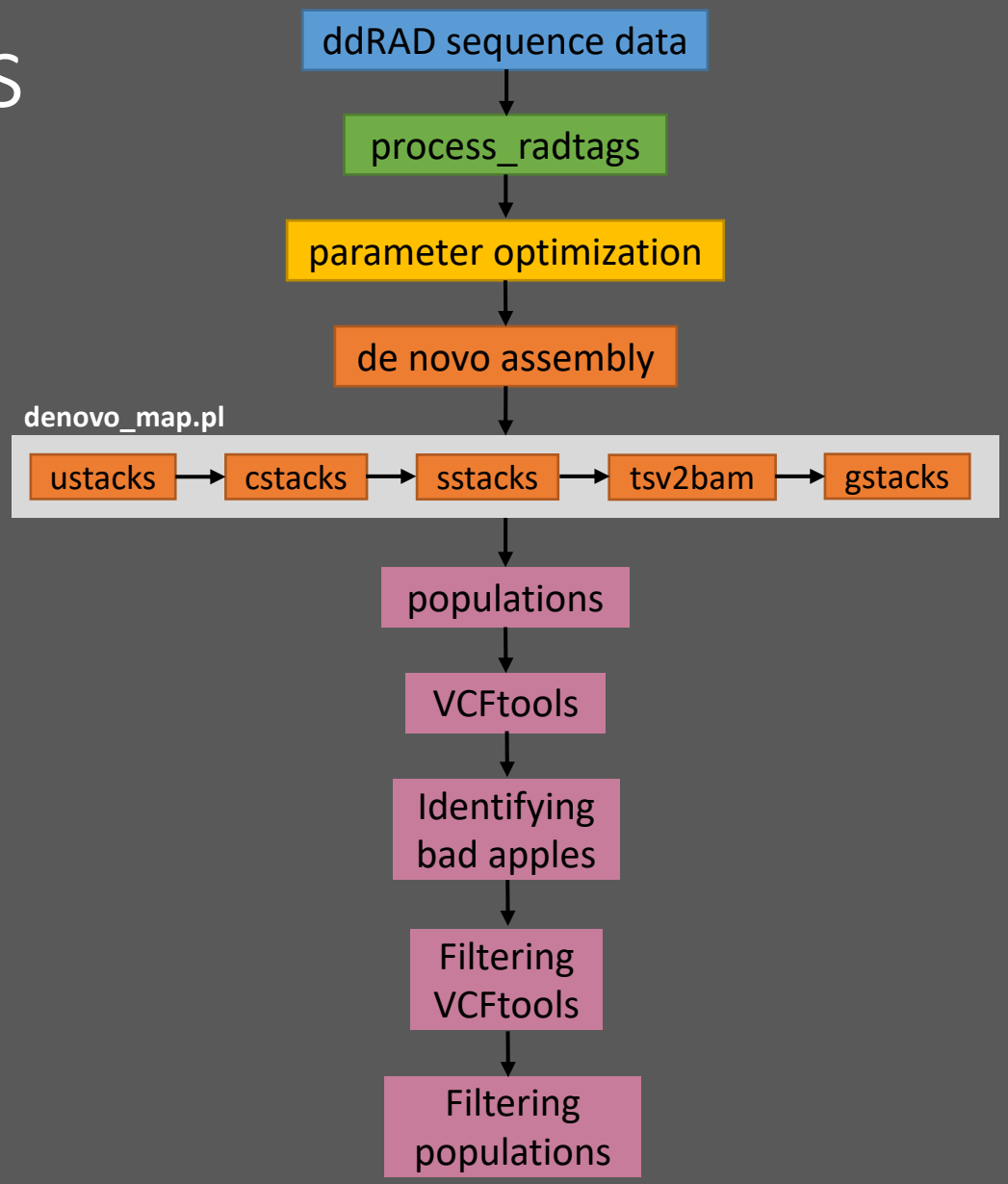
05_VCFtools_filtering

```bash
#!/bin/bash

#SBATCH -J vcftools_filtering
#SBATCH --mail-user=YOUREMAIL@gmail.com
#SBATCH --mail-type=BEGIN,END,FAIL,TIME_LIMIT
#SBATCH --output=/work/%u/%x-%j.out
#SBATCH --error=/work/%u/%x-%j.err
#SBATCH --mem-per-cpu=4G
#SBATCH -t 48:00:00

# Paths and filenames for this analysis

WORK_DIR="/work/$USER/ddRAD-seq_workshop"

out_dir="$WORK_DIR/outputs/Exercise_3/stacks.denovo/VCFtools"

vcf_dir="$WORK_DIR/outputs/Exercise_3/stacks.denovo/populations.snps.vcf"
log_file="$out_dir"/vcf_filtering_m5-100_miss0.25_2alleles.oe

## Load modules and activate software

module load foss/2019b VCFtools/0.1.16

# VCFtools - vcftools is a suite of functions for use on genetic variation data in the form of VCF and BCF files.
#The tools provided will be used mainly to summarize data, run calculations on data, filter out data, and convert data into other useful file formats.
# SYNOPSIS:
# vcftools [ --vcf FILE | --gzvcf FILE | --bcf FILE] [ --out OUTPUT PREFIX ] [ FILTERING OPTIONS ] [ OUTPUT OPTIONS ]

# Run VCFtools to filter the data

cd "$out_dir"
vcftools --vcf "$vcf_dir" --remove-indels --max-missing 0.50 --min-alleles 2 --max-alleles 2\
 --min-meanDP 5 --max-meanDP 100 --minDP 5 --maxDP 100 --recode --out "./filtered.m5-100_miss0.50_2alleles" &> "$log_file"
```

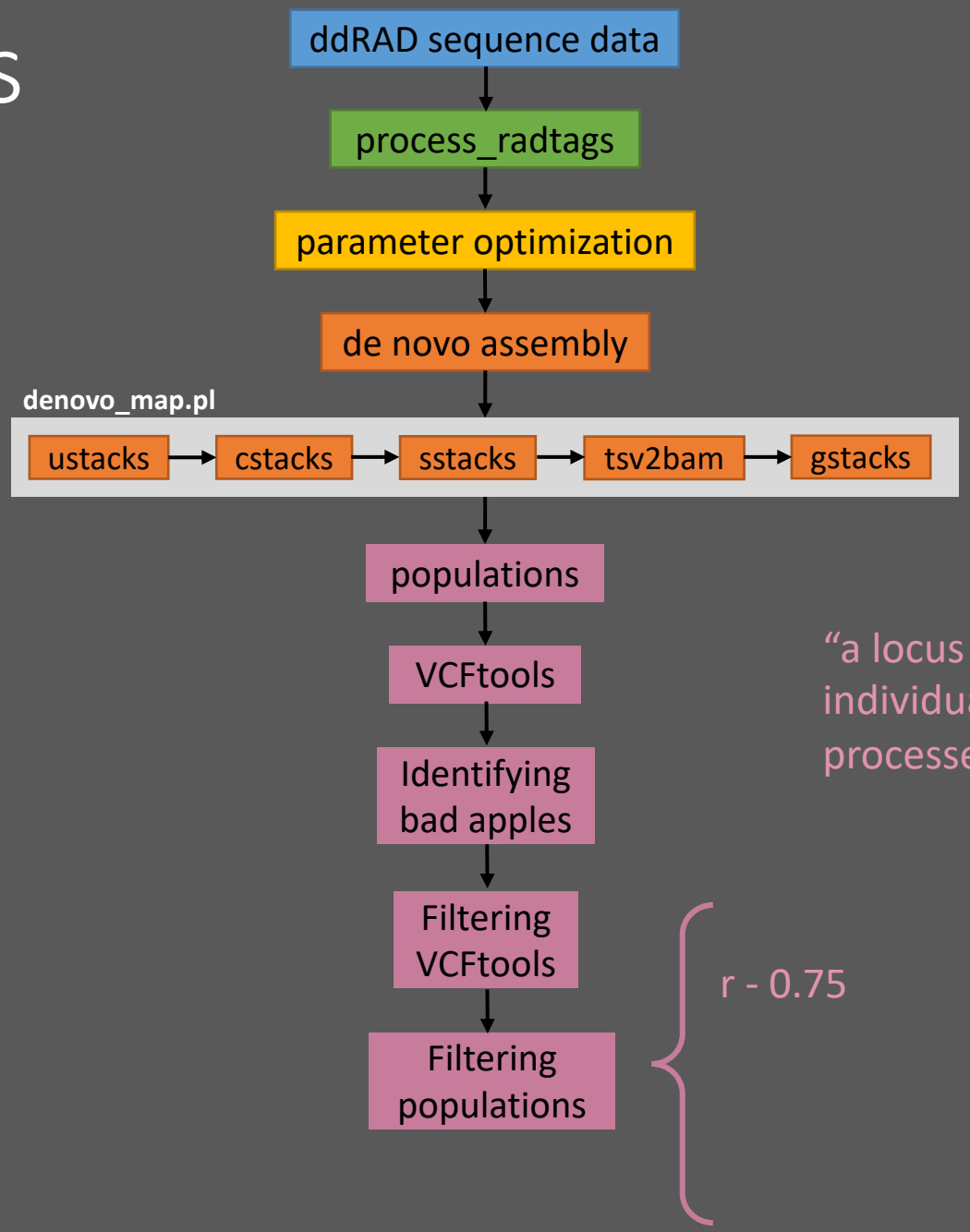
Bioinformatics Workflow



Bioinformatics Workflow

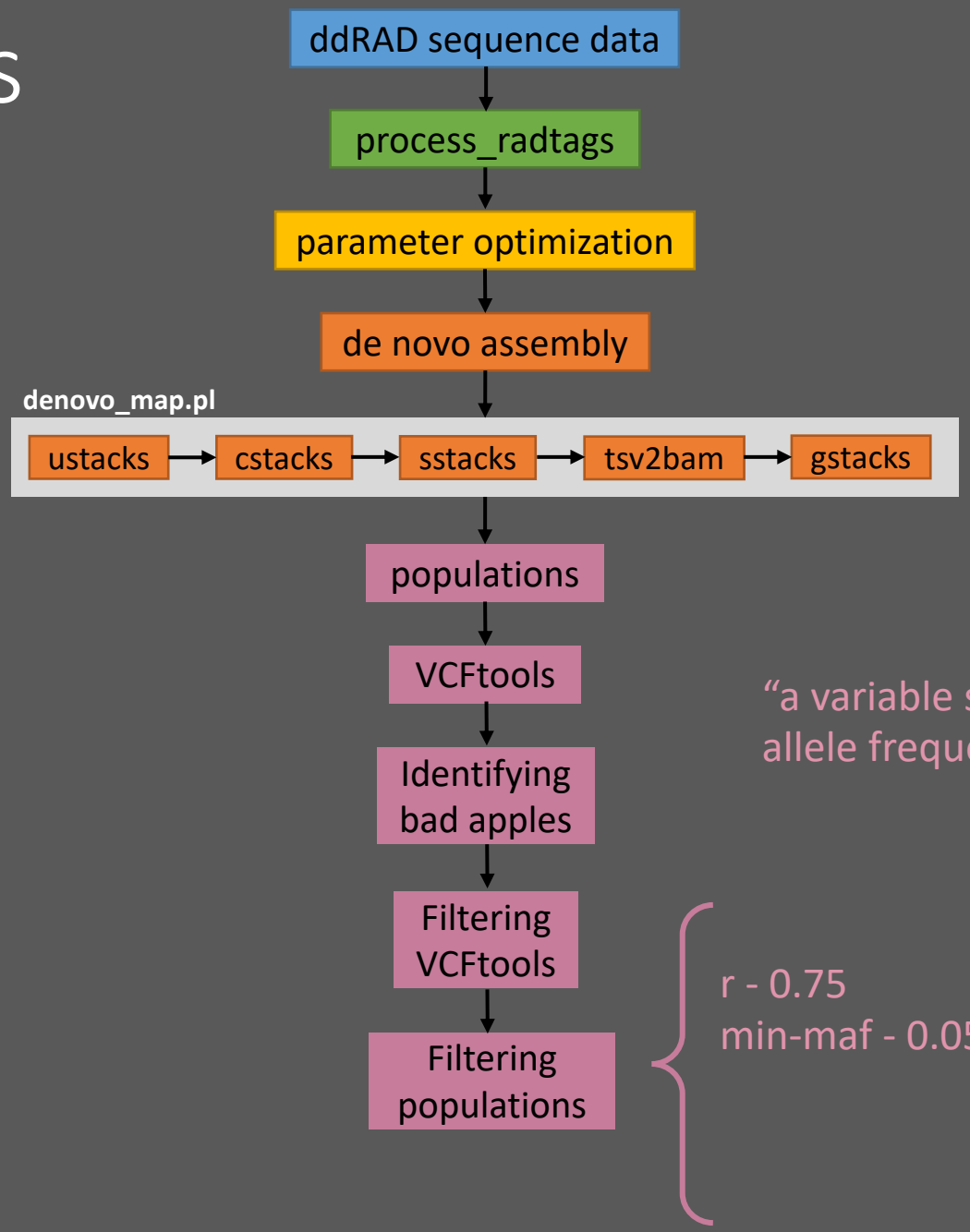


Bioinformatics Workflow



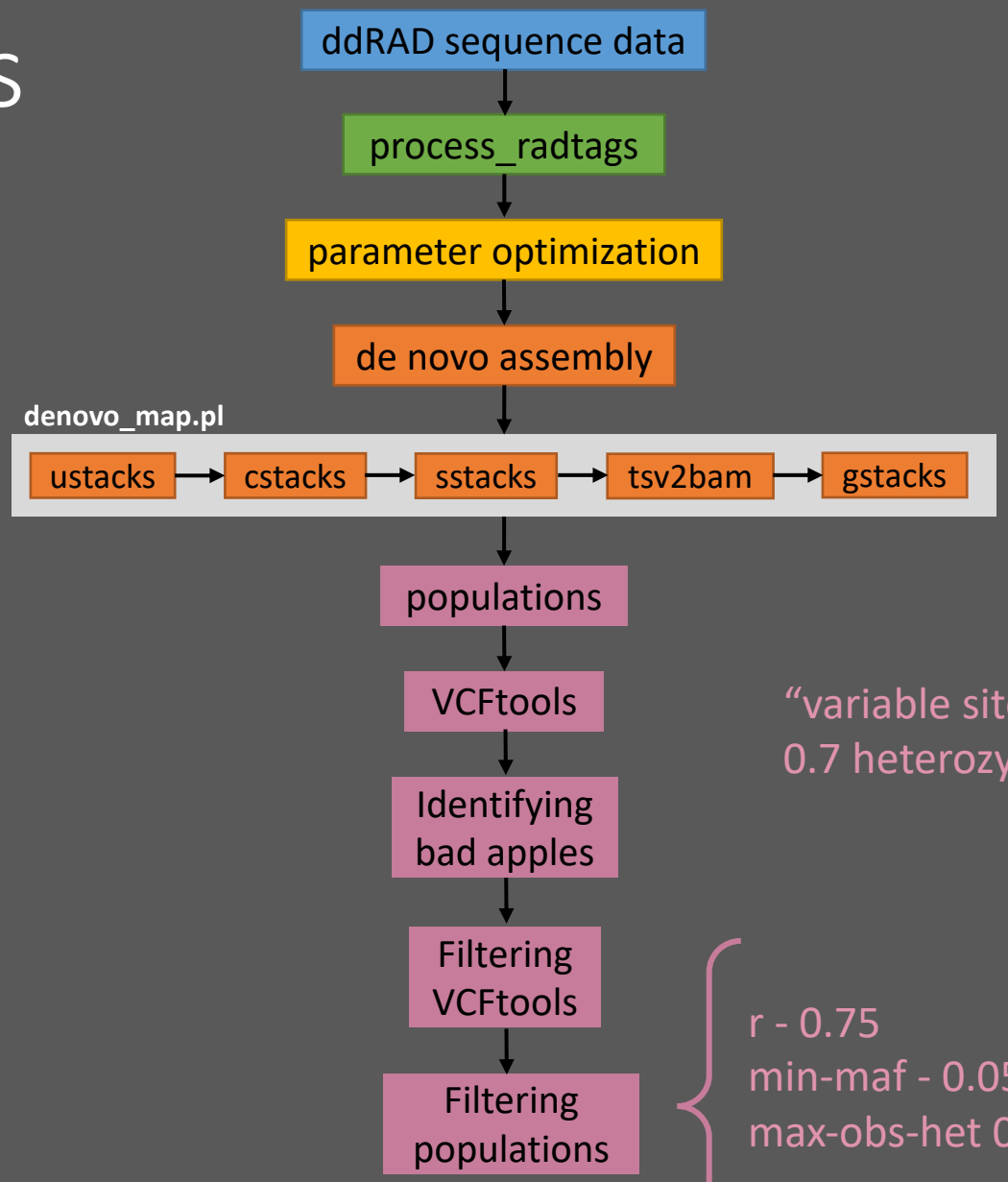
"a locus must be found in 75% of individuals of a single population to be processed"

Bioinformatics Workflow



"a variable site must possess a minimum allele frequency of 5% to be included"

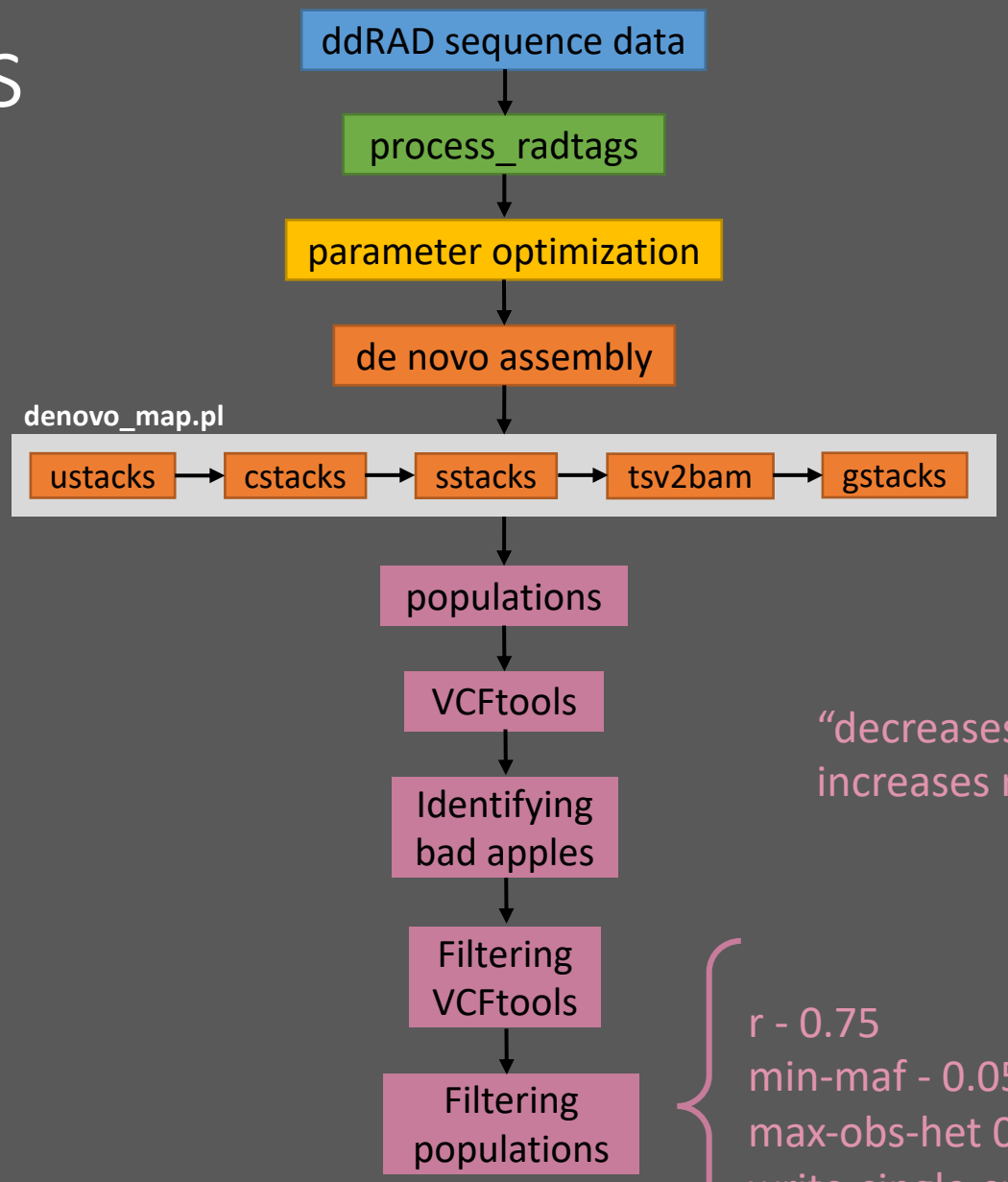
Bioinformatics Workflow



"variable sites with a maximum level of 0.7 heterozygosity will be included"

r - 0.75
min-maf - 0.05
max-obs-het 0.7

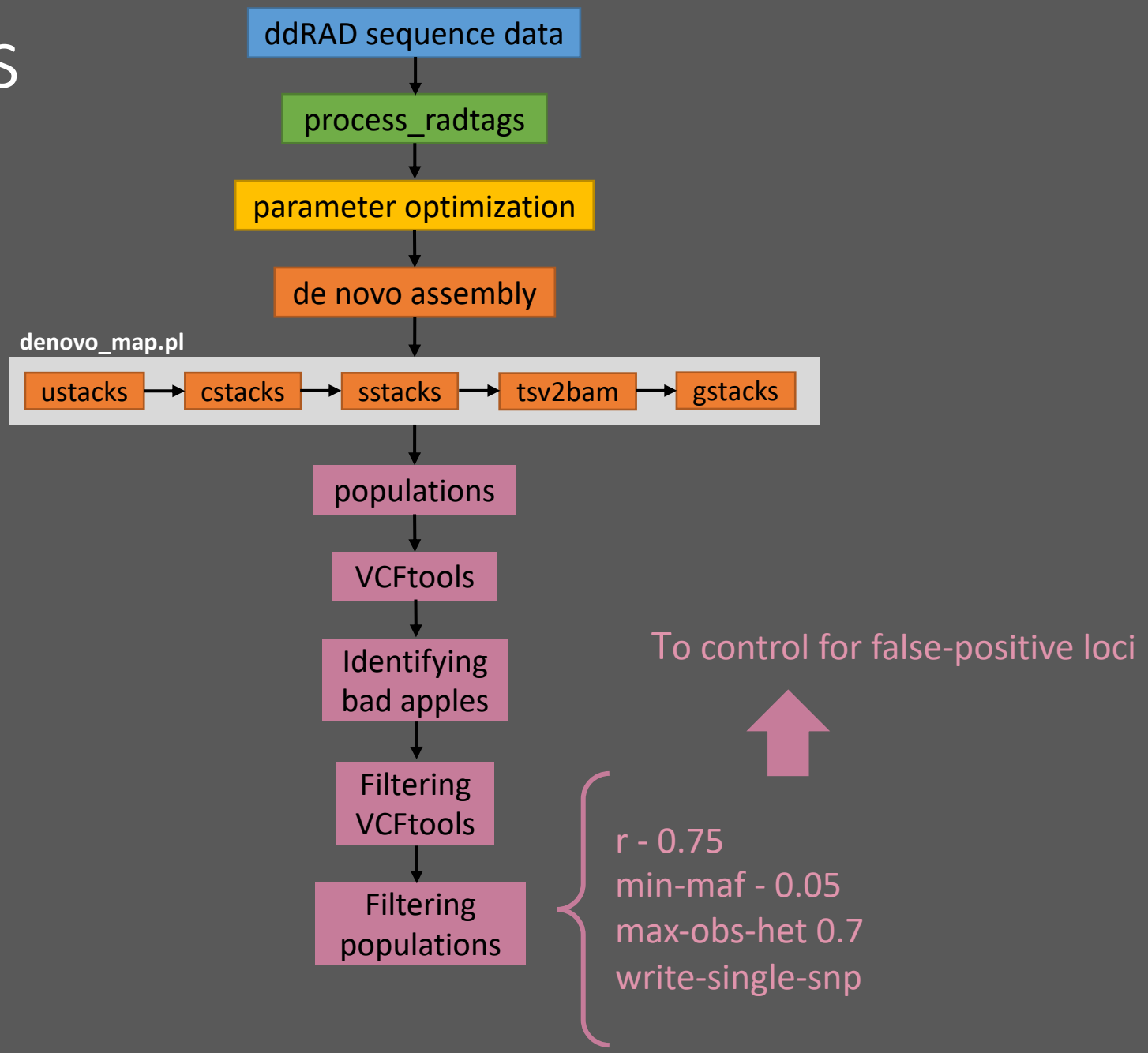
Bioinformatics Workflow



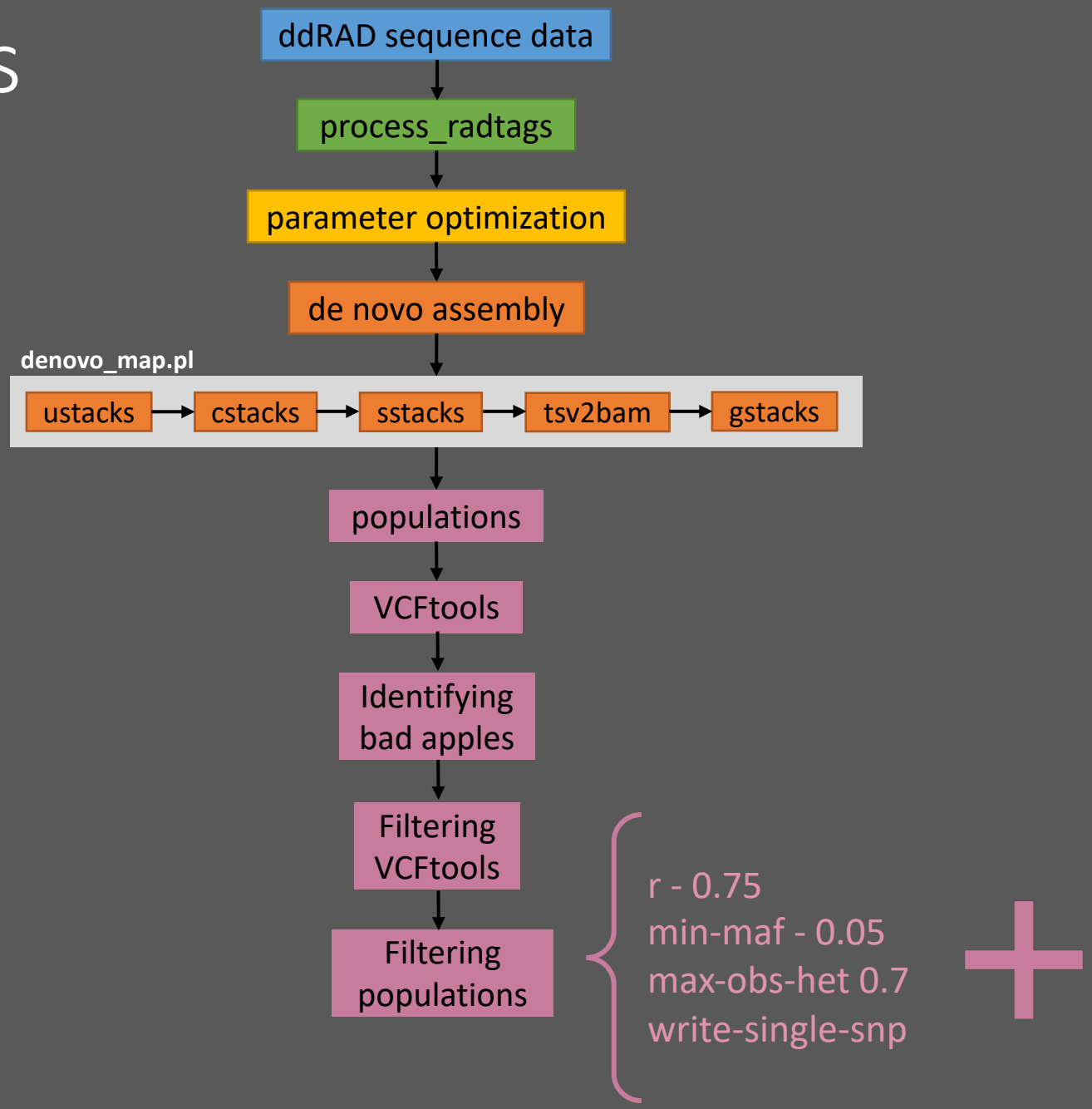
"decreases linkage disequilibrium and increases reproducibility"

r - 0.75
min-maf - 0.05
max-obs-het 0.7
write-single-snp

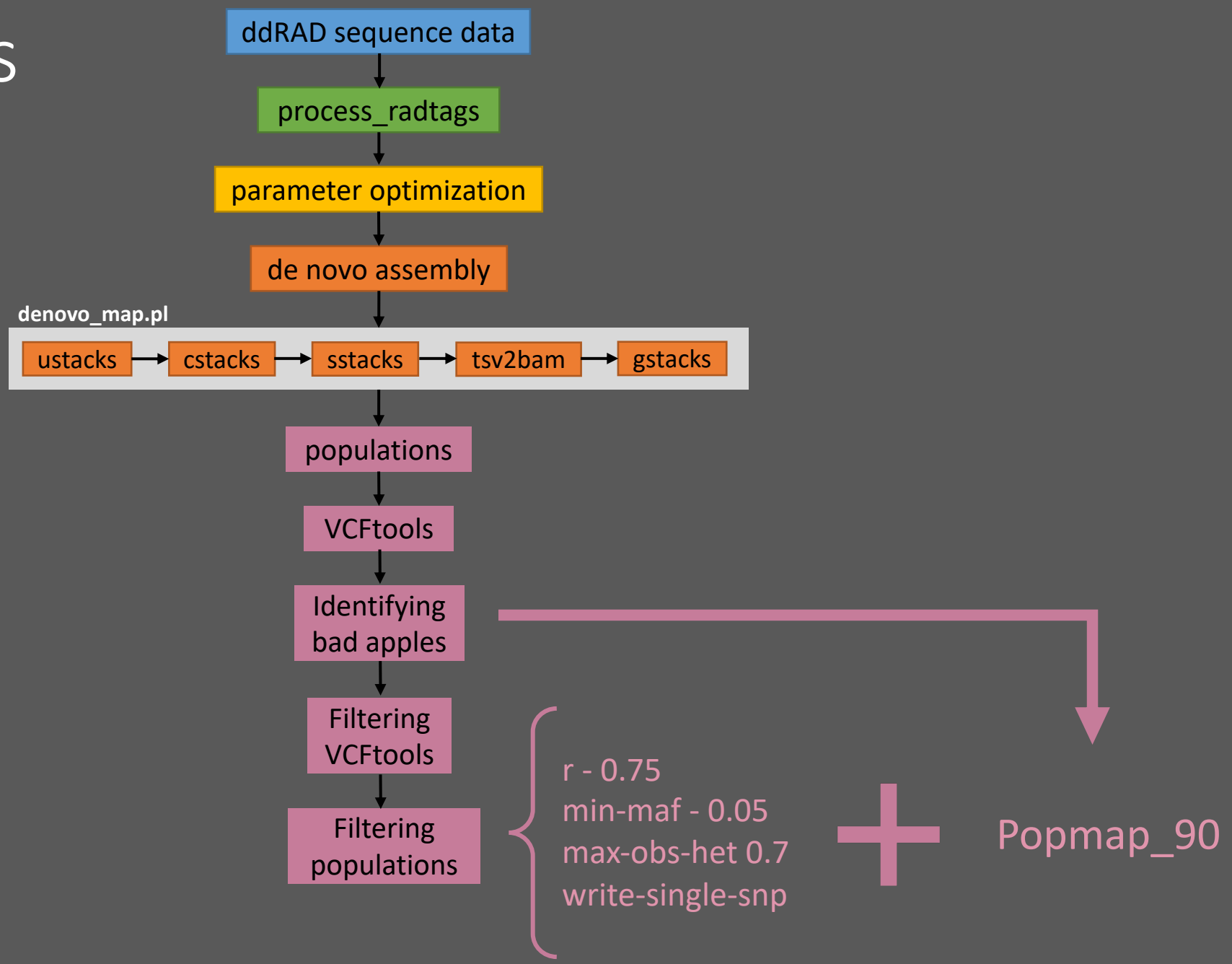
Bioinformatics Workflow



Bioinformatics Workflow



Bioinformatics Workflow



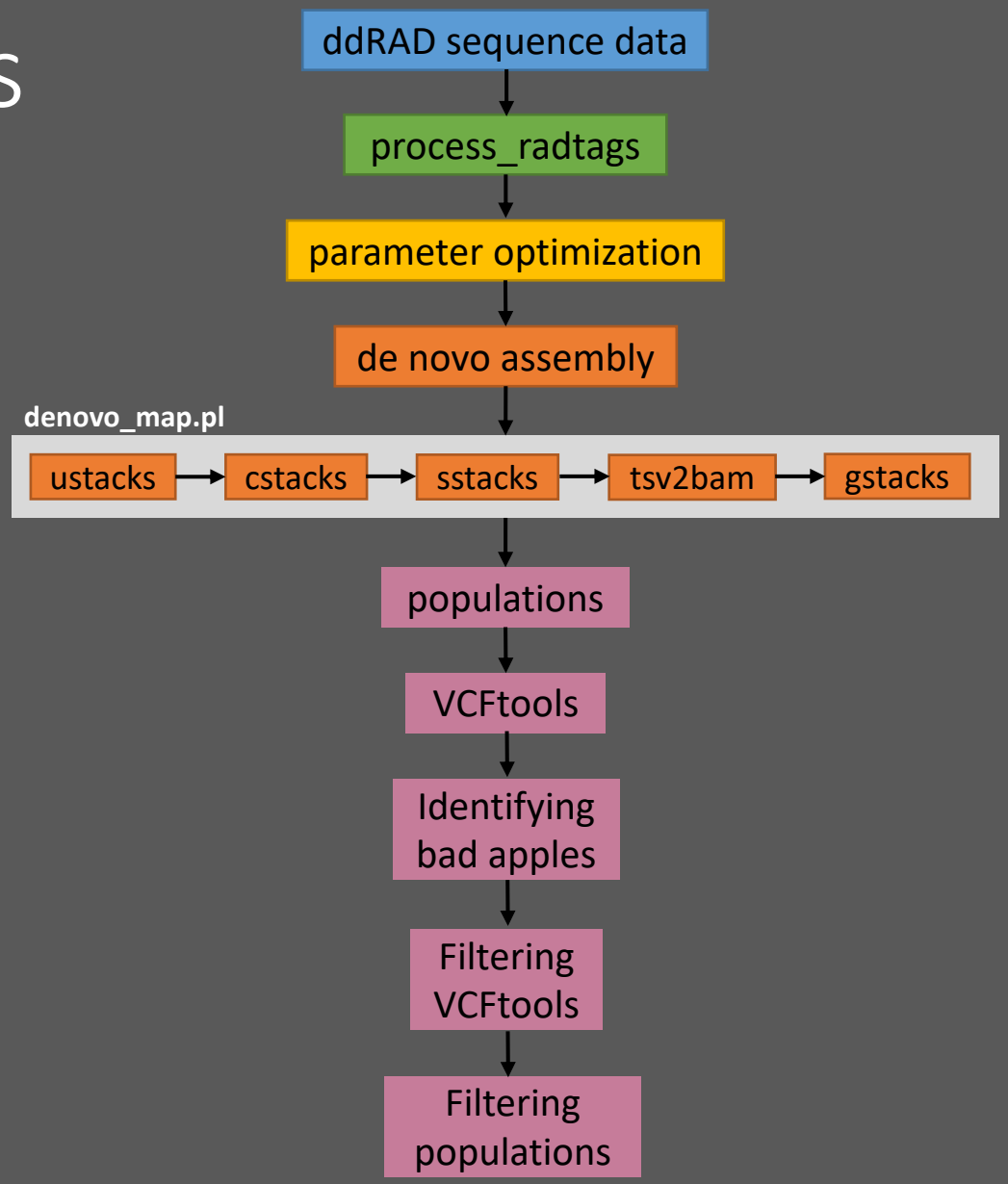
06_populations_filtering

```
# Paths and filenames for this analysis

WORK_DIR="/work/$USER/ddRAD-seq_workshop"

out_dir="$WORK_DIR/outputs/Exercise_3/stacks.denovo/populations.singleSNP.r075.m5.maf005.het07"
cd "$WORK_DIR" || exit
mkdir "$out_dir"
vcf_dir="$WORK_DIR/outputs/Exercise_3/stacks.denovo/VCFtools/filtered.m5-100_miss0.50_2alleles.recode.vcf"
popmap="/work/$USER/ddRAD-seq_workshop/data/Exercise_3/popmaps/popmap6.txt"
log_file="$out_dir"/populations.oe
```

06_populations_filtering

```
## Load modules and activate software

module load Anaconda3
source activate /data/Popgen/programs/stacks-2.53

# populations - it will analyze a population of individual samples computing a number of population genetics statistics
# as well as exporting a variety of standard output formats. A population map specifying which individuals belong to which
# population is submitted to the program and the program will then calculate population genetics statistics such as expected/observed
# heterozygosity, π, and FIS at each nucleotide position. The populations program will compare all populations pairwise to compute FST.
# The populations program provides strong filtering options to only include loci or variant sites that occur at certain frequencies in
# each population or in the metapopulation.

# -P = path to the directory containing the Stacks files (the gstacks output).
# --popmap = file path to the population map (<sample name><TAB><population>)
# -O = file path to write the pipeline output files
# -p = minimum number of populations a locus must be present in to process a locus.
# -m = coverage threshold
# -r = minimum percentage of individuals in a population required to process a locus for that population.
# --min-maf = specify a minimum minor allele frequency required to process a nucleotide site at a locus (0 < min_maf < 0.5).
# --write-single-snp = restrict data analysis to only the first SNP per locus.
# --write-random-snp = restrict data analysis to one random SNP per locus.
# --fstats – enable SNP and haplotype-based F statistics.
# -T = the number of threads/CPUs to use (default: 1)

# Run populations with "-r 0.75" (loci present in 75% of samples), min-maf 0.05 (a variable site must possess a minimum
# allele frequency of 5% to be included)
# --max-obs-het 0.7 (maximum level of heterozygosity a variable site can possess to be included) and writing only one
#single SNP (--write-single-snp).

populations -V "$vcf_dir" -O "$out_dir" --popmap "$popmap" \
-t "$SLURM_CPUS_PER_TASK" -r 0.75 --min-maf 0.05 --max-obs-het 0.7 \
--write-single-snp --fstats --hwe --vcf --plink --phylip --phylip-var --phylip-var-all &> "$log_file"
```

Bioinformatics Workflow



Bioinformatics Workflow