

SNPio: A Python API for Population Genetic File Processing, Filtering, and Encoding

Getting Started with SNPio

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Version: 1.1.0

2024-10-11

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SNPio: A Python API for Population Genetic File Processing, Filtering, and Encoding

Introduction

This guide provides an overview of how to get started with the SNPio library. It covers the basic steps to read, manipulate, and analyze genotype data using the `VCFReader`, `PhylipReader`, `StructureReader`, and `NRemover2` classes. SNPio is designed to simplify the process of handling genotype data and preparing it for downstream analysis, such as population genetics, phylogenetics, and machine learning. The library supports various file formats, including VCF, PHYLIP, and STRUCTURE, and provides tools for filtering, encoding, and visualizing genotype data. This guide will help you get up and running with SNPio quickly and efficiently.

`VCFReader`, `PhylipReader`, and `StructureReader` classes are used to read genotype data from VCF, PHYLIP, and STRUCTURE files, respectively. These classes load the data into a

`GenotypeData` object that has various useful methods and properties.

The `NRemover2` class is used to filter genotype data based on various criteria, such as missing data, minor allele count, minor allele frequency, and more. The `GenotypeEncoder` class is used to encode genotype data into different formats, such as one-hot encoding, integer encoding, and 0-1-2 encoding, for downstream analysis and machine learning tasks.

Below is a step-by-step guide to using SNPio to read, filter, and encode genotype data for analysis.

Installation

Before using SNPio, ensure it is installed in your Python environment. You can install it using pip. In the project root directory (the directory containing `setup.py`), type the following command into your terminal:

```
pip install snpio
```

We recommend using a virtual environment to manage your Python packages. If you do not have a virtual environment set up, you can create one using the following commands:

```
1 python3 -m venv snpio_env
   source snpio_env/bin/activate
```

This will create a virtual environment named `snpio_env` and activate it. You can then install SNPio in this virtual environment using the pip command mentioned above.

Note:

SNPio does not support Windows operating systems at the moment. We recommend using a Unix-based operating system such as Linux or macOS.

Note:

We aim to support anaconda environments in the future. For now, we recommend using a virtual environment with `pip` to install SNPio.

Importing SNPio

To start using SNPio, import the necessary modules:

```
# Import the necessary modules
2 from snpio import (
    NRemover2,
4    VCFReader,
    PhylipReader,
6    StructureReader,
    Plotting,
8    GenotypeEncoder,
)
```

Example usage:

```
1 # Define input filenames
  vcf = "example_data/vcf_files/phylogen_subset14K_sorted.vcf.gz"
3 popmap = "example_data/popmaps/phylogen_nomx.popmap"

5 # Load the genotype data from a VCF file
gd = VCFReader(
7     filename=vcf,
  popmapfile=popmap,
9     force_popmap=True,
  verbose=True,
11    plot_format="png",
  plot_fontsize=20,
13    plot_dpi=300,
  despine=True,
15    prefix="snpio_example"
)
```

You can also include or exclude any populations from the analysis by using the `include_pops` and `exclude_pops` parameters in the reader classes. For example:

```
# Only include the populations "ON", "DS", "EA", "GU", and "TT"
2 # Exclude the populations "MX", "YU", and "CH"
gd = VCFReader(
4     filename=vcf,
  popmapfile=popmap,
6     force_popmap=True,
  verbose=True,
8     plot_format="png",
  plot_fontsize=20,
10    plot_dpi=300,
  despine=True,
12    prefix="snpio_example",
  include_pops=["ON", "DS", "EA", "GU"],
14    exclude_pops=["MX", "YU", "CH"],
)
```

The `include_pops` and `exclude_pops` parameters are optional and can be used to filter the populations included in the analysis. If both parameters are provided, the populations in `include_pops` will be included, and the populations in `exclude_pops` will be excluded. However, populations cannot overlap between lists.

Important Notes

- The `VCFReader`, `PhylipReader`, `StructureReader`, `NRemover2`, and `GenotypeEncoder` classes treat the following characters as missing data:

- “N”
- “.”
- “?”
- “_”

- The `VCFReader` class can read both uncompressed and compressed VCF files (gzipped). If your input file is in PHYLIP or STRUCTURE format, it will be forced to be biallelic. To handle more than two alleles per site, use the VCF format.

The Population Map File

To use `VCFReader`, `PhylipReader`, or `StructureReader`, you can optionally use a population map (popmap) file. This is a simple two-column, whitespace-delimited or comma-delimited file with SampleIDs in the first column and the corresponding PopulationIDs in the second column. It can optionally contain a header line, with the first column labeled “SampleID” and the second column labeled “PopulationID” (case-insensitive). The population IDs can be any string, such as “Population1”, “Population2”, etc, or an integer. SampleIDs must match the sample names in the alignment file.

For example:

```
Sample1,Population1
2 Sample2,Population1
Sample3,Population2
4 Sample4,Population2
```

Or, with a header:

```
SampleID,PopulationID
2 Sample1,Population1
Sample2,Population1
4 Sample3,Population2
Sample4,Population2
```

The population map file is used to assign samples to populations and is useful for filtering and visualizing genotype data by population. If you do not provide a population map file, the samples will be treated as a single population.

The population map file can be provided as an argument to the reader classes. For example:

```
1 vcf = "example_data/vcf_files/phylogen_subset14K_sorted.vcf.gz"
  popmap = "example_data/popmaps/phylogen_nomx.popmap"
3
4 gd = VCFReader(
5     filename=vcf,
  popmapfile=popmap,
7     force_popmap=True,
  verbose=True,
```

```

9     plot_format="png",
      plot_fontsize=20,
11     plot_dpi=300,
      despine=True,
13     prefix="snpio_example"
  )

```

Note:

The `force_popmap` parameter in the reader classes is used to force the population map file to align with the samples in the alignment without an error. If set to **False**, the population map file must match the samples in the alignment exactly, and if they do not match, an error will be raised. If set to **True**, the population map file will be forced to align with the samples in the alignment by removing extra samples. This parameter is set to **False** by default.

The `verbose` parameter in the reader classes is used to print additional information about the genotype data and filtering steps.

The `plot_format`, `plot_fontsize`, `plot_dpi`, and `despine` parameters in the reader classes are used to customize the output plots generated by the reader classes. See API documentation for more details.

Reading Genotype Data

SNPio provides readers for different file formats. Here are examples of how to read genotype data from various file formats:

VCFReader

```

vcf = "example_data/vcf_files/phylogen_subset14K_sorted.vcf.gz"
2 popmap = "example_data/popmaps/phylogen_nomx.popmap"

4 gd = VCFReader(
      filename=vcf,
6     popmapfile=popmap,
      force_popmap=True,
8     verbose=True,
      plot_format="png",
10     plot_fontsize=20,
      plot_dpi=300,
12     despine=True,
      prefix="snpio_example",
14     exclude_pops=["MX", "YU", "CH"],
      include_pops=["ON", "DS", "EA", "GU", "TT"],
16 )

```

This will read the genotype data from a VCF file and apply the population map if provided.

PhylipReader

If you would like to read a Phylip file, you can use the `PhylipReader` class:

```
1 phylip = "example_data/phylip_files/phylogen_subset14K.phy"
2 popmap = "example_data/popmaps/phylogen_nomx.popmap"

4 gd = PhylipReader(
    filename=phylip,
6     popmapfile=popmap,
    force_popmap=True,
8     verbose=True,
    plot_format="png",
10    plot_fontsize=20,
    plot_dpi=300,
12    despine=True,
    prefix="snpio_example",
14    exclude_pops=["MX", "YU", "CH"],
    include_pops=["ON", "DS", "EA", "GU", "TT"],
16 )
```

StructureReader

If you would like to read in a Structure file, you can use the `StructureReader` class. For example:

```
1 structure =
    "example_data/structure_files/phylogen_subset14K.str"
2 popmap = "example_data/popmaps/phylogen_nomx.popmap"

4 gd = StructureReader(
    filename=structure,
6     popmapfile=popmap,
    force_popmap=True,
8     verbose=True,
    plot_format="png",
10    plot_fontsize=20,
    plot_dpi=300,
12    despine=True,
    prefix="snpio_example",
14    exclude_pops=["MX", "YU", "CH"],
    include_pops=["ON", "DS", "EA", "GU", "TT"],
16 )
```

Note:

The `StructureReader` class will automatically detect the format of the STRUCTURE

file. It can be in one-line or two-line format (see STRUCTURE documentation), and can optionally contain population information in the file as the second tab-delimited column. If the population information is not provided in the STRUCTURE file, you can provide a population map file to assign samples to populations.

Key Methods in VCFReader, PhylipReader, and StructureReader

`VCFReader(filename, popmapfile, force_popmap, ...)`: Reads and writes genotype data from/ to a VCF file and applies a population map if provided.

`write_vcf(output_file)`: Writes the filtered or modified genotype data back to a VCF file (for all three readers).

`PhylipReader(filename, popmapfile, force_popmap, ...)`: Reads and writes genotype data from/ to a PHYLIP file and applies a population map.

`write_phylip(output_file)`: Writes the filtered or modified genotype data back to a PHYLIP file (for PhylipReader).

`StructureReader(filename, popmapfile, force_popmap, ...)`: Reads and writes genotype data from/ to a STRUCTURE file and applies a population map.

`write_structure(output_file)`: Writes the filtered or modified genotype data back to a STRUCTURE file (for StructureReader).

Note:

The `write_vcf`, `write_phylip`, and `write_structure` methods are used to write the filtered or modified genotype data back to a VCF, PHYLIP, or STRUCTURE file, respectively. **These methods can also be used to convert between file VCF, PHYLIP, and STRUCTURE formats.**

Other GenotypeData Methods

The `GenotypeData` along with the `Plotting` classes have several useful methods for working with genotype data:

- `Plotting.run_pca()`: Runs principal component analysis (PCA) on the genotype data and plots the results. The PCA plot can help visualize the genetic structure of the populations in the dataset, with each point representing an individual. Individuals are colored by missing data proportion, and populations are represented by different shapes. A 2-dimensional PCA plot is generated by default, but you can specify three PCA axes as well. For example:
- `GenotypeData.missingness_reports()`: Generates missing data reports and plots for the dataset. The reports include the proportion of missing data per individual, per locus, and per population. These reports can help you identify samples, loci, or populations with high levels of missing data. For example:

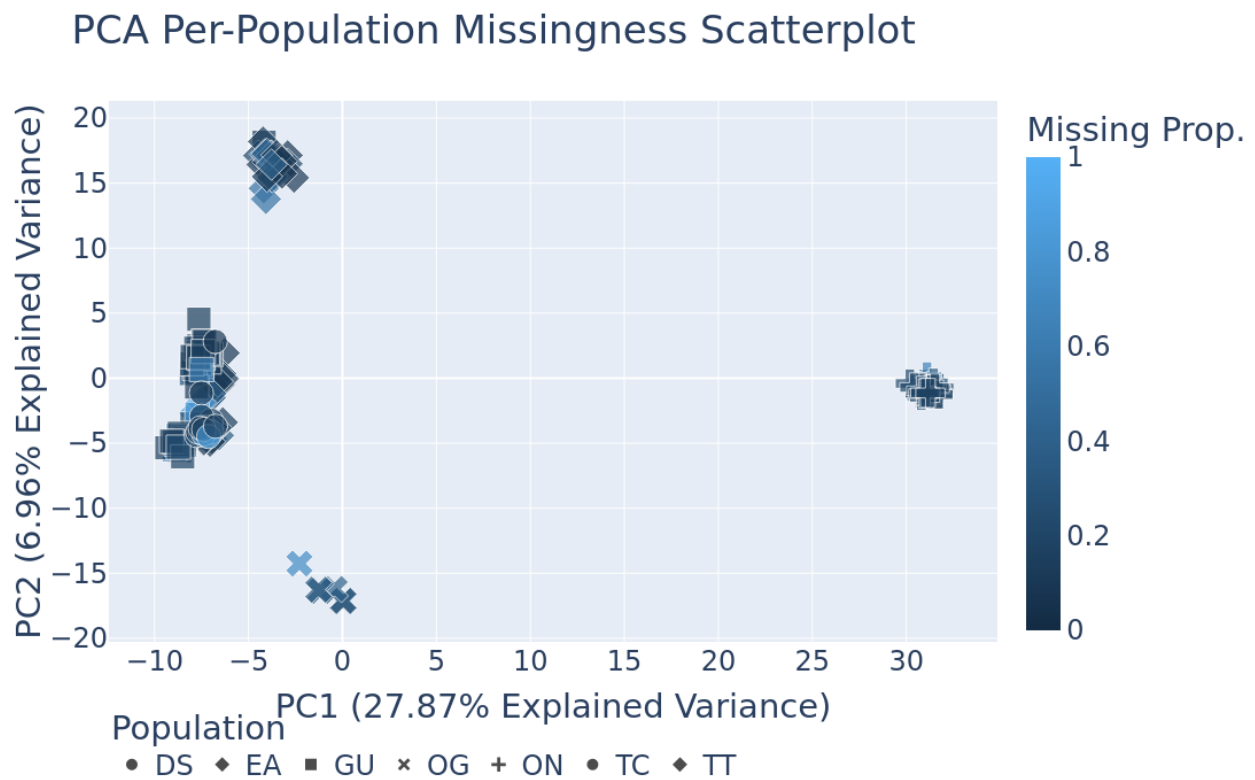


Figure 1: Principle Component Analysis (PCA) colored by missingness proportion. Shapes depict distinct populations.

Missingness Report

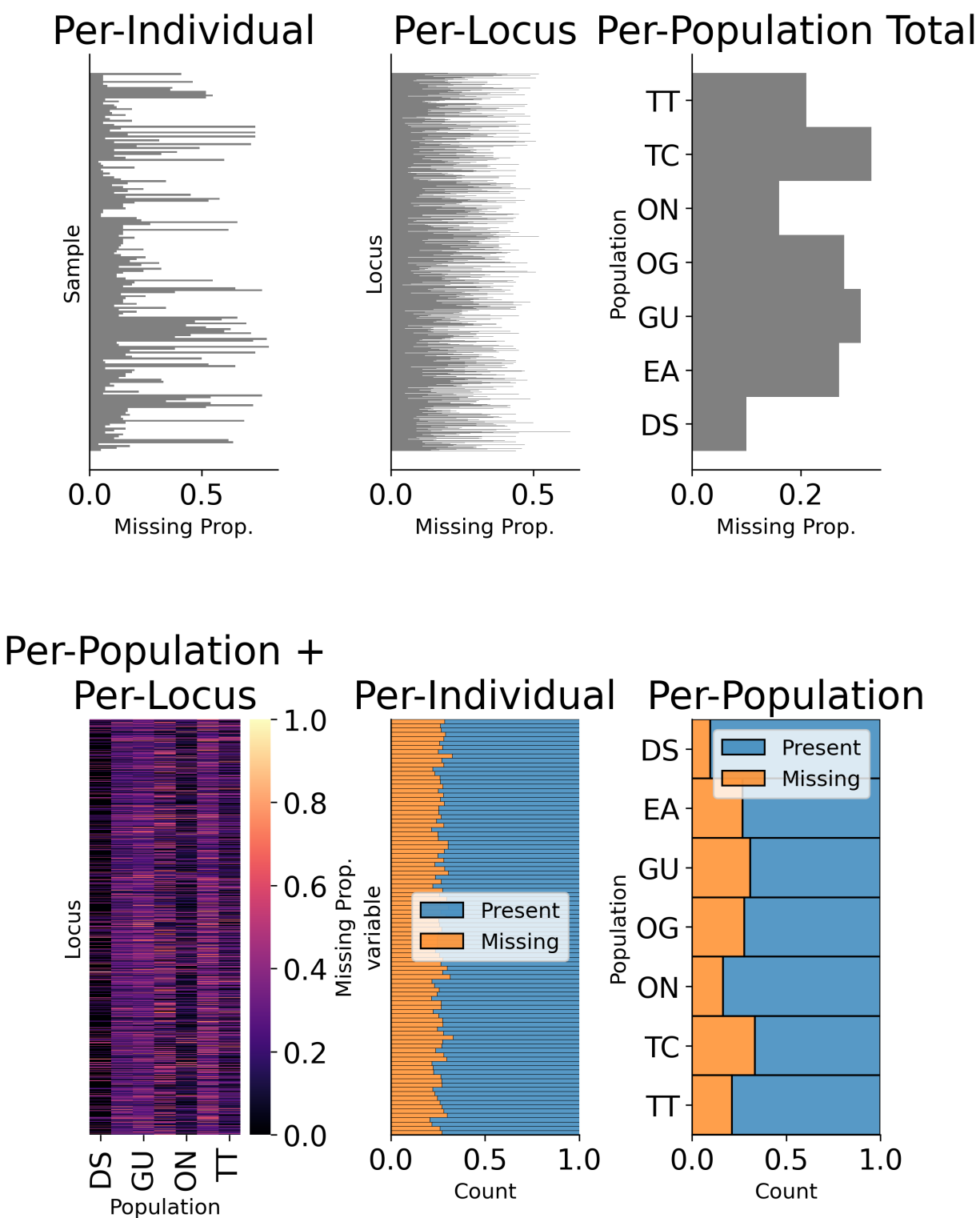


Figure 2: Plots depicting missing data proportions for samples and loci (SNPs).

- The `GenotypeData` class will automatically create a plot showing the number of individuals present in each population, if a `popmapfile` is provided. For example:

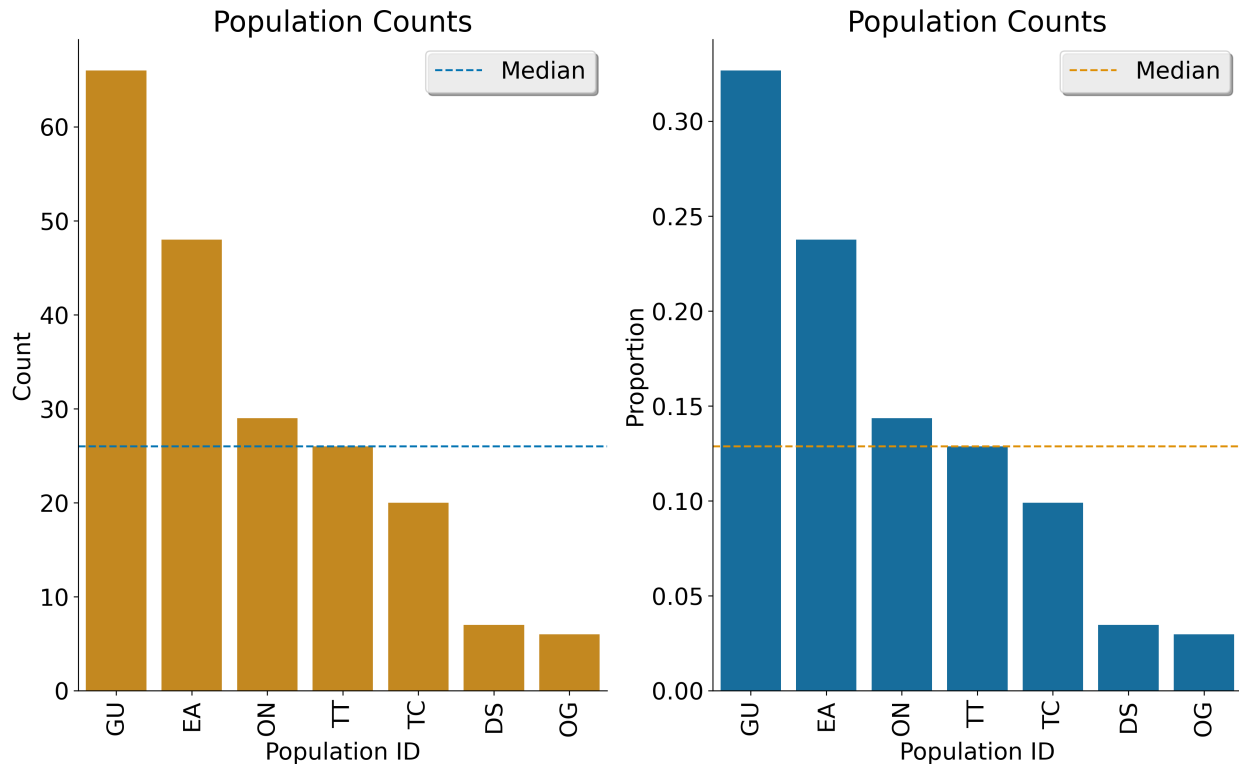


Figure 3: Bar plot depicting counts per populations as provided in the population map file.

Filtering Genotype Data with NRemover2

NRemover2 provides a variety of filtering methods to clean your genotype data. Here is an example of how to apply filters to remove samples and loci with too much missing data, monomorphic sites, singletons, minor allele count (MAC), minor allele frequency (MAF), and more:

```
# Apply filters to remove samples and loci with too much
missing data
2 gd_filt = nrm.filter_missing_sample(0.75)
      .filter_missing(0.75)
4      .filter_missing_pop(0.75)
      .filter_mac(2)
6      .filter_monomorphic(exclude_heterozygous=False)
      .filter_singletons(exclude_heterozygous=False)
8      .filter_biallelic(exclude_heterozygous=False)
      .resolve()
10
# Write the filtered VCF to a new file
12 gd_filt.write_vcf("filtered_output.vcf")
```

Key Methods in NRemover2

`filter_missing_sample(threshold)`: Filters samples with missing data above the threshold.

`filter_missing(threshold)`: Filters loci with missing data above the threshold.

`filter_missing_pop(threshold)`: Filters loci where missing data for any given population is above the threshold.

`filter_mac(threshold)`: Filters loci with a minor allele count below the threshold.

`filter_maf(threshold)`: Filters loci with a minor allele frequency below the threshold.

`filter_monomorphic(exclude_heterozygous)`: Filters monomorphic loci (sites with only one allele).

`filter_singletons(exclude_heterozygous)`: Filters singletons (sites with only one occurrence of an allele).

`filter_biallelic(exclude_heterozygous)`: Filters biallelic loci (sites with only two alleles).

`thin_loci(size)`: Thins loci by removing loci within `size` bases of each other on the same locus or chromosome (based on input VCF `CHROM` and `POS` fields). Note that this method only works with `VCFReader` and is not available for `PhylipReader` and `StructureReader`. For example, `thin_loci(100)` will remove all but one locus within 100 bases of each other on the same chromosome.

`filter_linked(size)`: Filters loci that are linked to other loci within a specified distance (`size`), only considering the `CHROM` field from the VCF file and ignoring the `POS` field. This method only works with `VCFReader` and is not available for `PhylipReader` and `StructureReader`.

`random_subset_loci(size)`: Randomly selects `size` number of loci from the input dataset, where `size` is an integer.

`resolve()`: Applies the filters and returns the filtered `GenotypeData` object. This method must be called at the end of the filtering chain to apply the filters.

Note:

You must call `resolve()` at the end of the filtering chain to apply the filters and return the filtered `GenotypeData` object.

Note:

The `exclude_heterozygous` parameter in `filter_monomorphic`, `filter_singletons`, and `filter_biallelic` methods allows you to exclude heterozygous genotypes from the filtering process. By default, heterozygous genotypes are included in the filtering process.

Note:

`thin_loci` and `filter_linked` are only available for `VCFReader` and not for `PhylipReader` and `StructureReader`.

Warning:

The `filter_linked(size)` method might yield a limited number of loci with SNP data. It is recommended to use this method with caution and check the output carefully.

Additional Methods in `NRemover2`

`search_thresholds()` searches a range of filtering thresholds for all missing data, minor allele frequency (MAF), and minor allele count (MAC) filters. This method helps you find the optimal thresholds for your dataset. It will plot the threshold search results so you can visualize the impact of different thresholds on the dataset.

With `search_thresholds()`, you can specify the thresholds to search for and the order in which to apply the filters:

```
# Initialize NRemover2 with GenotypeData object
2 nrm = NRemover2(gd)

4 # Specify filtering thresholds and order of filters
nrm.search_thresholds(
6     thresholds=[0.25, 0.5, 0.75, 1.0],
    maf_thresholds=[0.01, 0.05],
8     mac_thresholds=[2, 5],
    filter_order=[
10         "filter_missing_sample",
        "filter_missing",
12         "filter_missing_pop",
        "filter_mac",
14         "filter_monomorphic",
        "filter_singletons",
16         "filter_biallelic"
    ]
18 )
```

The `search_thresholds()` method will search for the optimal thresholds for missing data, MAF, and MAC filters based on the specified thresholds and filter order. It will plot the results so you can visualize the impact of different thresholds on the dataset.

Below are example plots that are created when running the `search_thresholds()` method:

Filtering Results for Singletons, Monomorphic Sites, and Biallelic Sites:

Filtering Results for Minor Allele Count (MAC):

Filtering Results for Minor Allele Frequency:

Missing Data Filtering for Loci and Samples:

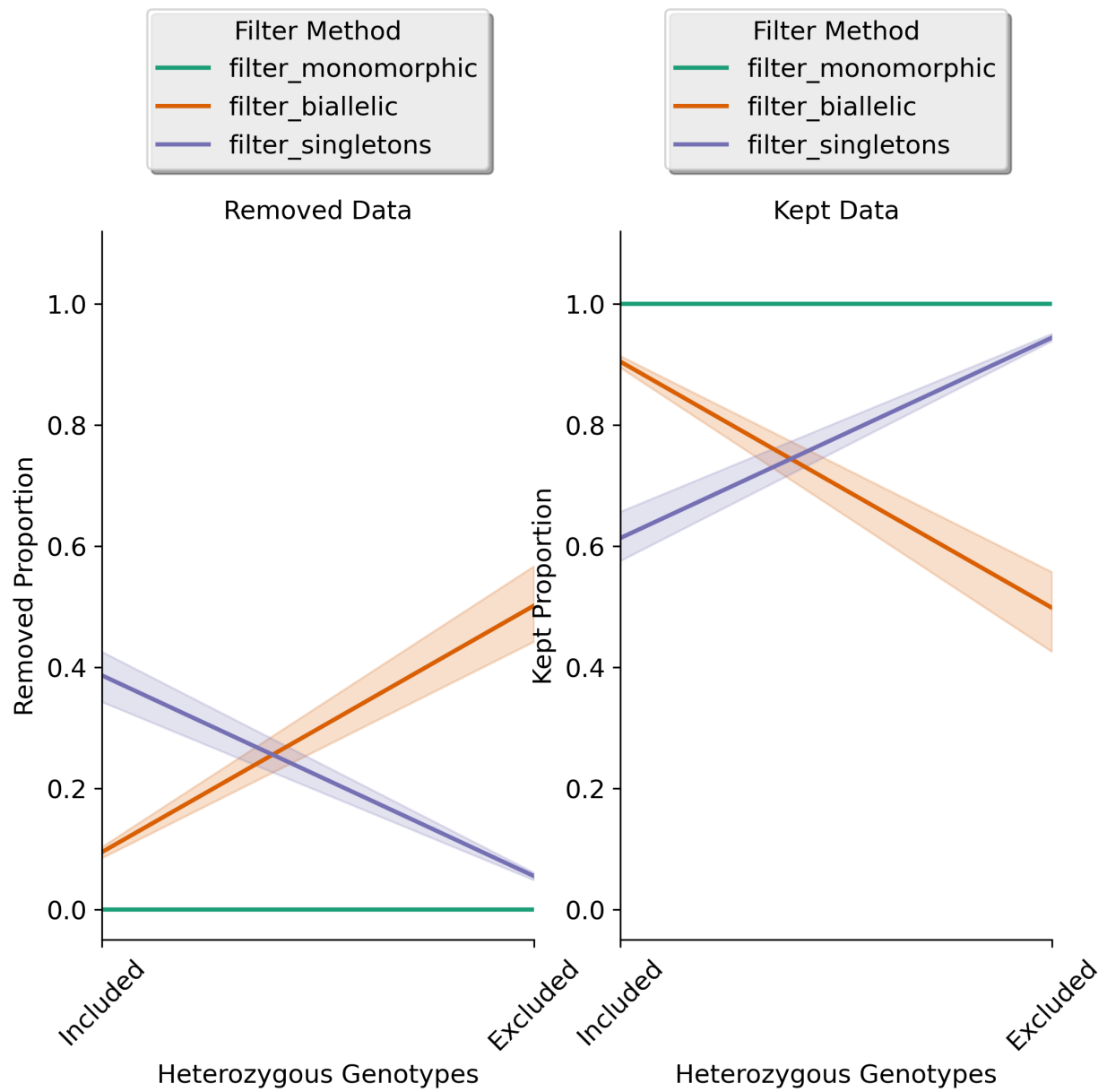


Figure 4: NRemove2 filtering results for the boolean filtering methods ('filter_monomorphic', 'filter_singletons', and 'filter_biallelic')

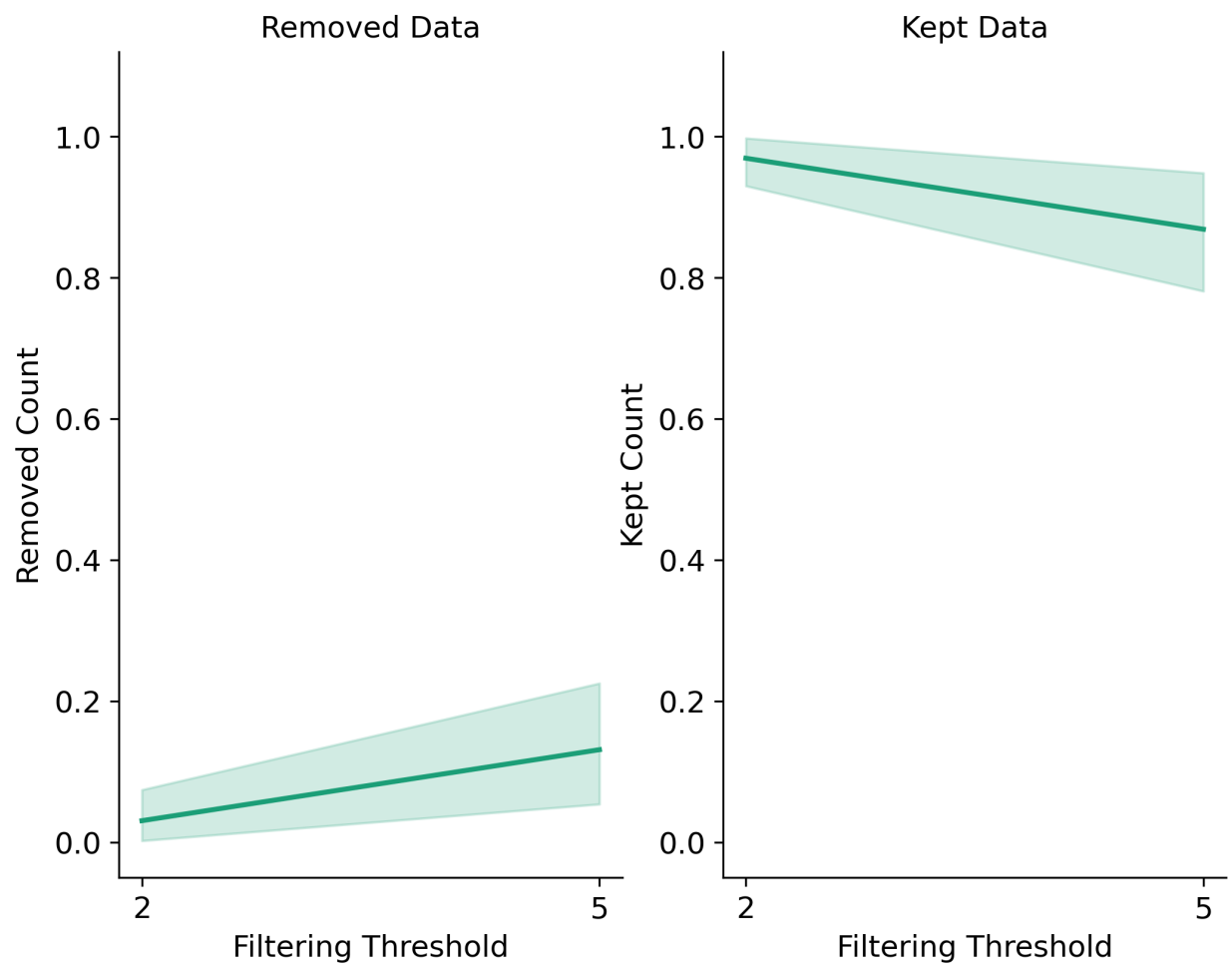


Figure 5: NRemove2 filtering results for the Minor Allele Count filtering method

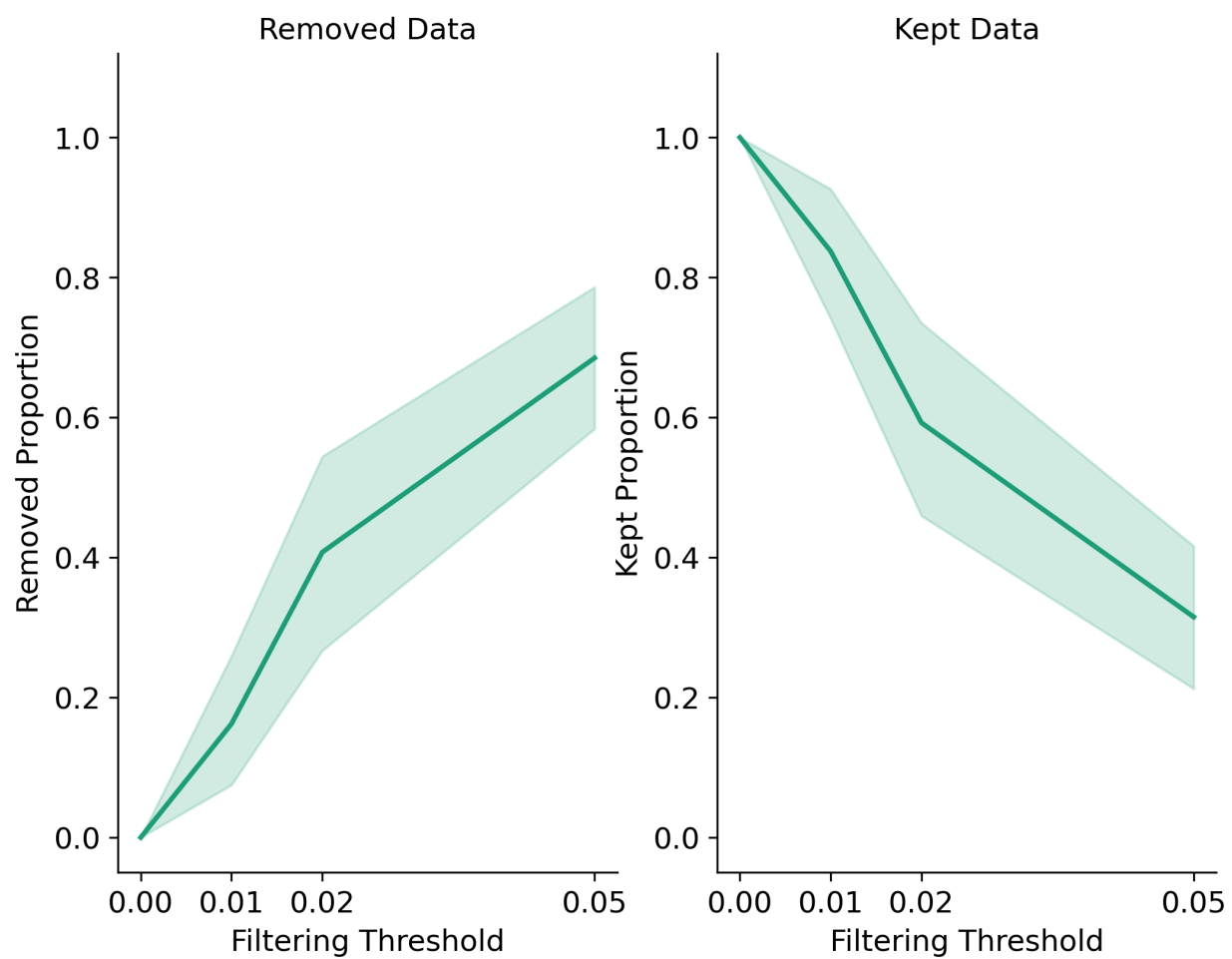


Figure 6: NRemover2 filtering results for the Minor Allele Frequency method

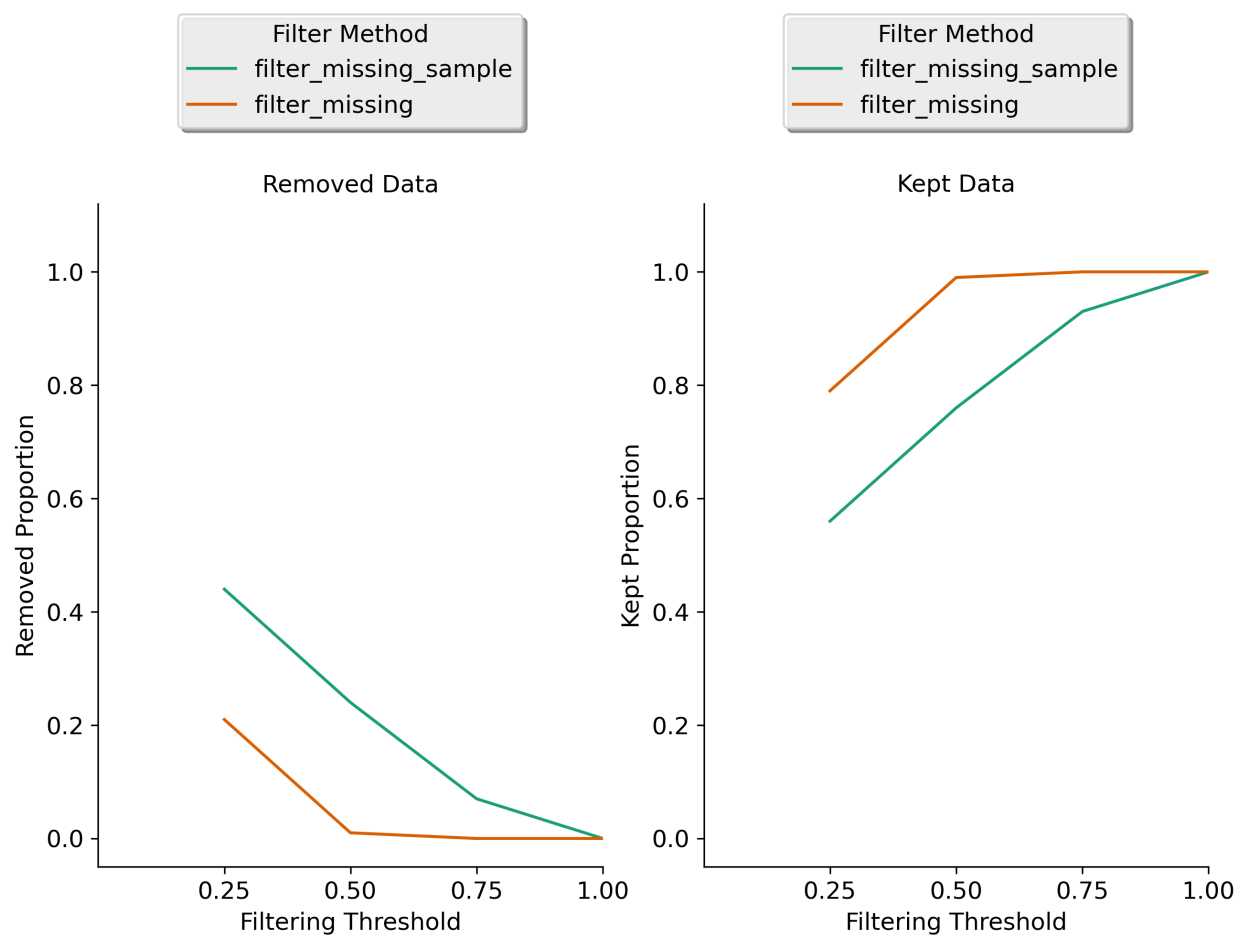


Figure 7: NRemover2 filtering results for the missing data methods ('filter_missing' and 'filter_missing_samples'). The 'filter_missing' method filters out columns (loci) exceeding a missing data threshold, whereas the 'filter_missing_sample' method filters out samples (rows) exceeding the threshold.

Missing Data Filtering for Populations:

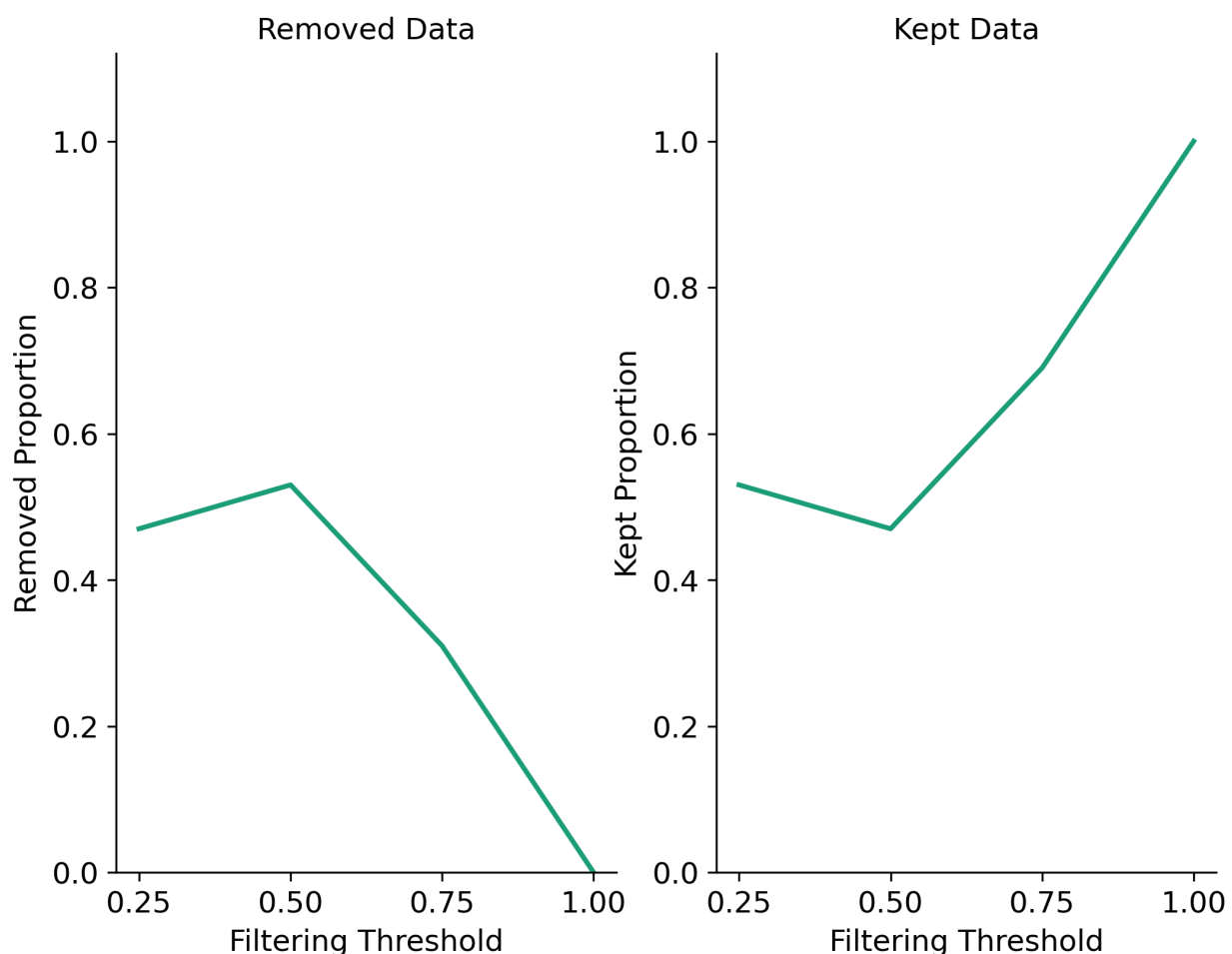


Figure 8: NRemove2 filtering results for the ‘filter_missing_pop’ method, which filters out loci (SNP columns) wherein any given population group exceeds the provided missing data threshold.

Note:

The `search_thresholds()` method is incompatible with `thin_loci(size)` and `filter_linked()` being in the `filter_order` list.

Warning:

The `search_thresholds()` method can also be called either before or after any other filtering, but note that it will reset the filtering chain to the original state.

`plot_sankey_filtering_report()` generates a Sankey plot to visualize how SNPs are filtered at each step of the pipeline. For example:

```
from snpio import NRemove2, VCFReader
vcf = "example_data/vcf_files/phylogen_subset14K_sorted.vcf.gz"
```

```

4 popmap = "example_data/popmaps/phylogen_nomx.popmap"

6 gd = VCFReader(
    filename=vcf,
8     popmapfile=popmap,
    force_popmap=True,
10    verbose=True,
    plot_format="png",
12    plot_fontsize=20,
    plot_dpi=300,
14    despine=True,
    prefix="snpio_example"
16 )

18 # Initialize NRemover2.
    nrm = NRemover2(gd)
20
    # Apply filters to remove samples and loci.
22 gd_filt = nrm.filter_missing_sample(0.75)
    .filter_missing(0.75)
24    .filter_missing_pop(0.75)
    .filter_mac(2)
26    .filter_monomorphic(exclude_heterozygous=False)
    .filter_singletons(exclude_heterozygous=False)
28    .filter_biallelic(exclude_heterozygous=False)
    .resolve()
30
    nrm.plot_sankey_filtering_report()

```

This will automatically track the number of loci at each filtering step and generate a Sankey plot to visualize the filtering process. The Sankey plot shows how many loci are removed at each step of the filtering process. For example:

In the Sankey Diagram above, the green nodes represent the number of loci remaining after each filtering step, and the red nodes represent the number of loci removed at each filtering step. The size of each edge is proportional to the number of loci retained or removed at each step. The Sankey plot provides a visual representation of the filtering process and helps you understand how each filtering method affects the dataset. The filtering order is dynamic based on the order each method was called.

Note:

The `plot_sankey_filtering_report()` must be called after filtering and calling the `resolve()` method to generate the Sankey plot. It is also incompatible with `thin_loci()`, `filter_linked()`, and `random_subset_loci()` being in the `filter_order` list.

`plot_sankey_filtering_report()` only plots loci removed at each filtering step and does

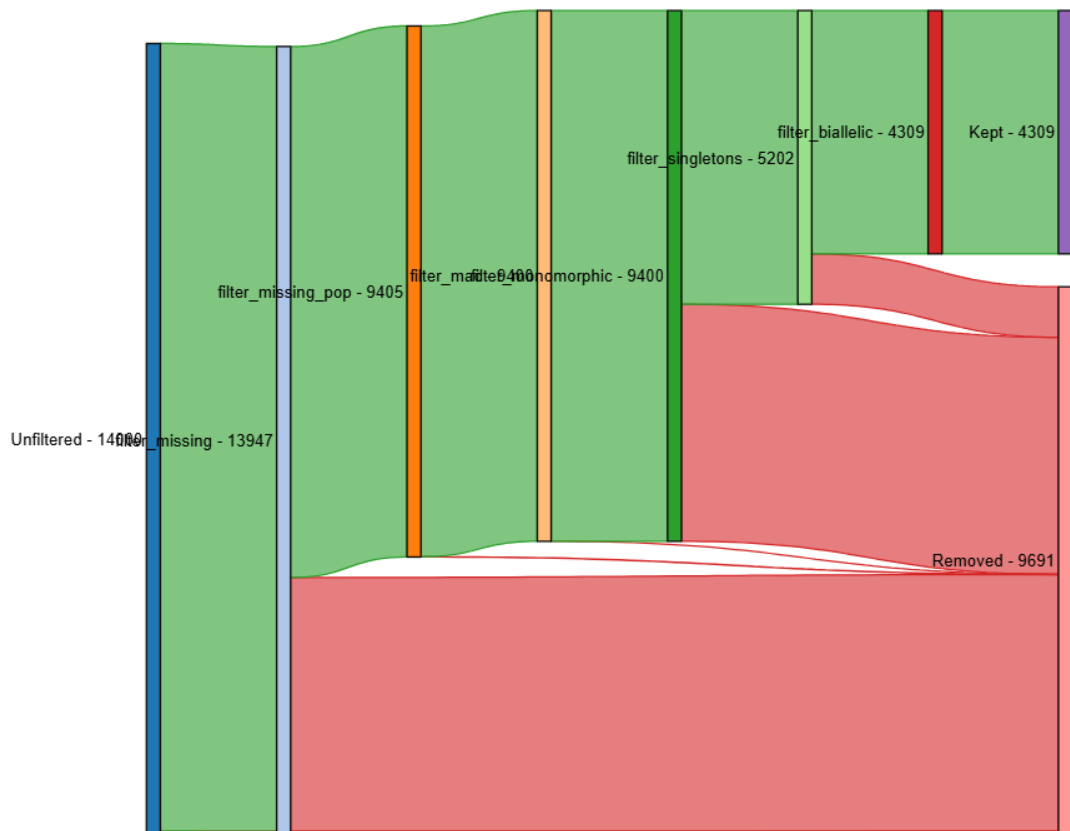


Figure 9: Sankey Diagram depicting the number (count) of loci retained (green bands) and removed (red bands) at each NRemove2 filtering step. Band widths are proportional to the number of loci retained and removed at each consecutive step.

not plot samples removed.

GenotypeData Properties

Once genotype data is loaded using any of the readers, you can access several useful properties from the **GenotypeData** object:

num_snps: Number of SNPs or loci in the dataset.

num_inds: Number of individuals in the dataset.

populations: List of populations in the dataset.

popmap: Mapping of SampleIDs to PopulationIDs.

popmap_inverse: Dictionary with population IDs as keys and lists of samples as values.

samples: List of samples in the dataset.

snpsdict: Dictionary with sampleIDs as keys and genotypes as values.

loci_indices: Numpy array with boolean values indicating the loci that passed the filtering criteria set to **True**.

sample_indices: Numpy array with boolean values indicating the samples that passed the filtering criteria set to **True**.

snp_data: 2D numpy array of SNP data of shape (num_inds, num_snps).

ref: List of reference alleles for each locus.

alt: List of alternate alleles for each locus.

inputs: Dictionary of input parameters used to load the genotype data.

Genotype Encoding with GenotypeEncoder

SNPio also includes the **GenotypeEncoder** class for encoding genotype data into formats useful for downstream analysis and commonly used for machine and deep learning tasks.

The **GenotypeEncoder** class provides three encoding properties:

genotypes_onehot: Encodes genotype data into one-hot encoding, where each possible biallelic IUPAC genotype is represented by a one-hot vector. Heterozygotes are represented as multi-label vectors as follows:

```
onehot_dict = {
2  "A": [1.0, 0.0, 0.0, 0.0],
   "T": [0.0, 1.0, 0.0, 0.0],
4  "G": [0.0, 0.0, 1.0, 0.0],
   "C": [0.0, 0.0, 0.0, 1.0],
6  "N": [0.0, 0.0, 0.0, 0.0],
   "W": [0.5, 0.5, 0.0, 0.0],
```

```

8  "R": [0.5, 0.0, 0.5, 0.0],
   "M": [0.5, 0.0, 0.0, 0.5],
10 "K": [0.0, 0.5, 0.5, 0.0],
   "Y": [0.0, 0.5, 0.0, 0.5],
12 "S": [0.0, 0.0, 0.5, 0.5],
   "N": [0.0, 0.0, 0.0, 0.0],
14 }

```

genotypes_int: Encodes genotype data into integer encoding, where each possible biallelic IUPAC genotype is represented by an integer as follows: as follows: A=0, T=1, G=2, C=3, W=4, R=5, M=6, K=7, Y=8, S=9, N=-9.

genotypes_012: Encodes genotype data into 0-1-2 encoding, where 0 represents the homozygous reference genotype, 1 represents the heterozygous genotype, and 2 represents the homozygous alternate genotype.

Example Usage:

```

from snpio import VCFReader, GenotypeEncoder
2
vcf = "example_data/vcf_files/phylogen_subset14K_sorted.vcf.gz"
4 popmap = "example_data/popmaps/phylogen_nomx.popmap"

6 gd = VCFReader(
    filename=vcf,
8    popmapfile=popmap,
    force_popmap=True,
10   verbose=True,
    plot_format="png",
12   plot_fontsize=20,
    plot_dpi=300,
14   despine=True,
    prefix="snpio_example"
16 )

18 encoder = GenotypeEncoder(gd)

20 # Convert genotype data to one-hot encoding
gt_ohc = encoder.genotypes_onehot
22
24 # Convert genotype data to integer encoding
gt_int = encoder.genotypes_int

26 # Convert genotype data to 0-1-2 encoding.
gt_012 = encoder.genotypes_012

```

The GenotypeEncoder allows you to seamlessly convert genotype data into different formats depending on your needs for analysis or machine learning workflows.

You can also inversely convert the encoded data back to the original genotypes by just setting the GenotypeEncoder properties to a new value. For example:

```
1 # Convert one-hot encoded data back to genotypes
  encoder.genotypes_onehot = gt_ohe
3
  # Convert integer encoded data back to genotypes
5 encoder.genotypes_int = gt_int

7 # Convert 0-1-2 encoded data back to genotypes
  encoder.genotypes_012 = gt_012
```

This will automatically update the original genotype data in the GenotypeData object and convert it to the original format stored in the `snp_data` property of the GenotypeData object.

Benchmarking the Performance

You can benchmark the filtering performance using the Benchmark class to visualize how thresholds affect the dataset, if you have installed the snpio dev requirements:

```
pip install snpio[dev]
```

Then, you can use the Benchmark class to plot performance metrics for your filtered genotype data after the `resolve()` method is called. For example:

```
from snpio.utils.benchmarking import Benchmark
2
Benchmark.plot_performance(
4     nrm.genotype_data,
    nrm.genotype_data.resource_data
6 )
```

This function will plot performance metrics for your filtered genotype data and for the VCFReader class, giving insights into data quality changes.

For more information on the Benchmark class and how to use it, see the API documentation.

Conclusion

This guide provides an overview of how to get started with the SNPio library. It covers the basic steps to read, manipulate, and analyze genotype data using the VCFReader, PhylipReader, StructureReader, and NRemover2 classes. SNPio is designed to simplify the process of handling genotype data and preparing it for downstream analysis, such as population genetics, phylogenetics, and machine learning. The library supports various file formats, including VCF, PHYLIP, and STRUCTURE, and provides tools for filtering,

encoding, and visualizing genotype data. This guide will help you get up and running with SNPio quickly and efficiently.

For more information on the SNPio library, please refer to the API documentation and examples provided in the repository. If you have any questions or feedback, please feel free to reach out to the developers. We hope you find SNPio useful for your genotype data analysis tasks!

Note:

The SNPio library is under active development, and we welcome contributions from the community. If you would like to contribute to the project, please check the GitHub repository for open issues and submit a pull request. We appreciate your support and feedback!

If you encounter any issues or have any questions about the SNPio library, please feel free to reach out to the developers or open an issue on the GitHub repository. We are here to help and improve the library based on your feedback.

The SNPio library is licensed under the GPL3 License, and we encourage you to use it for your research and analysis tasks. If you find the library useful, please cite it in your publications. We appreciate your support and feedback!