GenoPHI

Genotype-to-Phenotype Phage-Host Interaction Prediction

GenoPHI is a Python package for machine learning-based prediction of genotype-phenotype relationships using whole-genome sequence data. Originally designed for phage-host interaction prediction, GenoPHI supports both binary interaction prediction and regression tasks for any microbial phenotype. The package implements protein family-based and *k*-mer-based approaches to extract genomic features from amino acid sequences and predict phenotypes using CatBoost gradient boosting models.

Workflow Overview

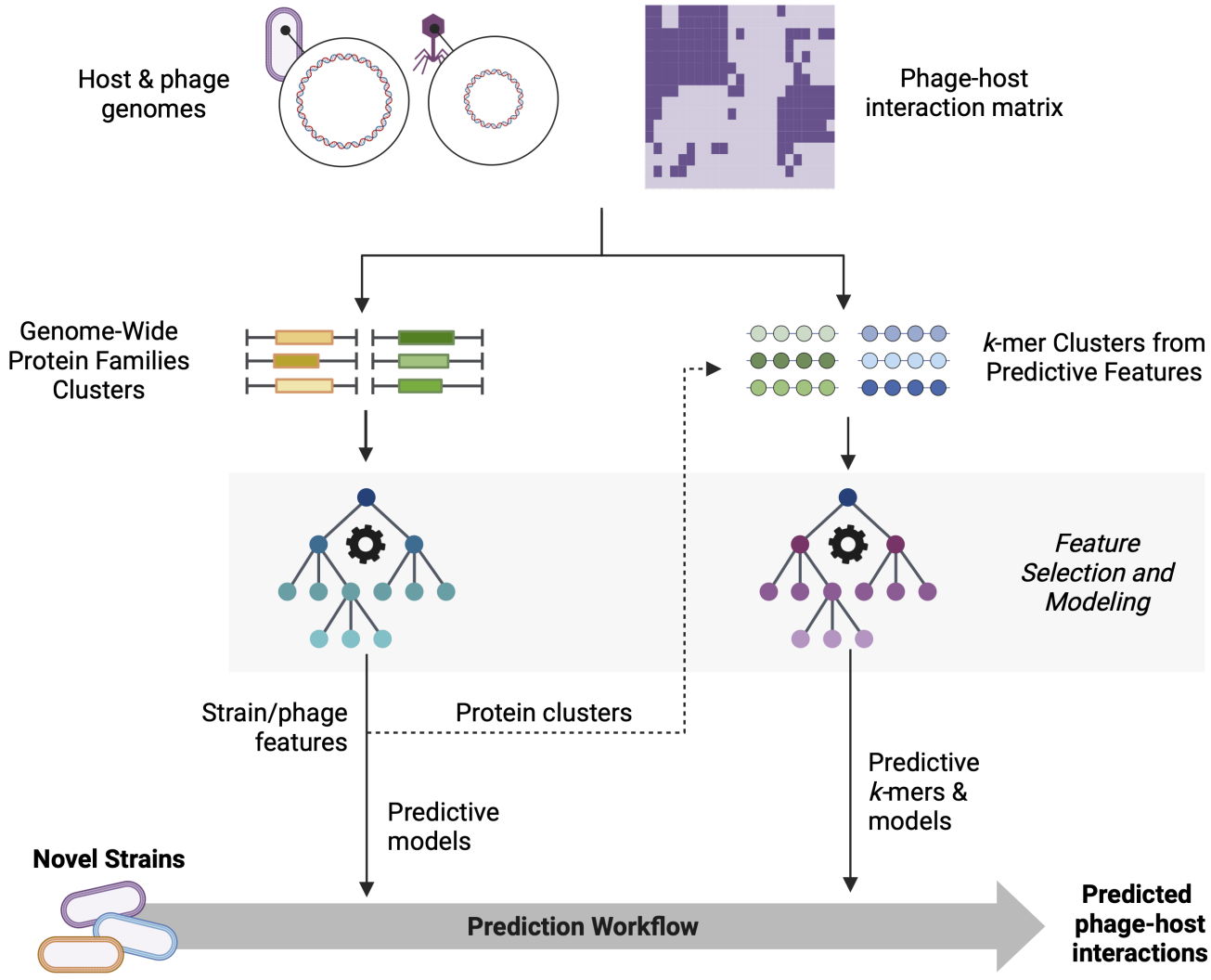

Figure 1: GenoPHI workflow schematic showing the main analysis pipelines: Protein family-based workflow, K-mer-based workflow, and Predictive protein k-mer workflow. Each pathway includes feature extraction, selection, model training, and prediction steps.

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Features

Protein Family-Based Analysis

1. **MMSeqs2 Clustering**: Cluster protein sequences into protein families based on sequence similarity
2. **Feature Table Generation**: Create presence-absence matrices of protein families across genomes and consolidate into predictive features based on co-occurence across genomes
3. **Feature Selection**: Identify predictive protein families (multiple available methods: RFE, SHAP, SHAP-RFE, ANOVA, Chi-squared, Lasso)
4. **Model Training**: Train CatBoost models with hyperparameter optimization
5. **Phenotype Prediction**: Predict interactions, resistance, or other phenotypes for new genomes
6. **Feature Annotation**: Identify predictive protein sequences from predictive features

K-mer-Based Analysis

1. ***K*-mer Feature Extraction**: Generate *k*-mer features from protein sequences with or without gene family context
2. **Predictive *K*-mer Workflow**: Extract *k*-mers specifically from predictive protein families identified in protein family analysis
3. **Feature Selection & Modeling**: Apply same robust feature selection and modeling pipelines
4. **Flexible *K*-mer Lengths**: Support for single *k*-mer length or ranges (e.g., 3-6)

Application Modes

- **Phage-Host Interaction Prediction**: Binary prediction of infection outcomes between phages and bacterial strains
- **Single-Strain Phenotype Prediction**: Predict strain-level phenotypes (e.g., antibiotic resistance, growth rate) without requiring phage data

- **Regression Tasks**: Predict continuous phenotypes (e.g., infection efficiency, metabolic rates)
- **General Feature-Based Modeling**: Use any feature table with a phenotype column for custom applications

Advanced Capabilities

- **Dynamic Feature Weighting**: Account for feature frequency distributions to handle imbalanced features
- **Clustering-Based Selection**: Use HDBSCAN or hierarchical clustering for intelligent feature grouping
- **Multiple Feature Selection Methods**: RFE, SHAP-RFE, SelectKBest, Chi-squared, Lasso, SHAP
- **Comprehensive Performance Metrics**: AUC-ROC, Precision-Recall, MCC, F1-score, Accuracy
- **SHAP Interpretability**: Feature importance analysis and visualization for model explainability
- **Bootstrapping Support**: Robust model evaluation with multiple train-test splits

Installation

Prerequisites

External Dependency: GenoPHI requires MMseqs2 for protein sequence clustering and assignment.

Install via conda/mamba:

```
conda install -c bioconda mmseqs2
# or
mamba install -c bioconda mmseqs2
```

For other installation methods, see the MMSeqs2 Wiki.

System Requirements

Minimum Requirements:

- Python 3.8 or higher
- 8 GB RAM
- 4 CPU cores
- 10 GB free disk space

Recommended for Large Datasets:

- Python 3.10+
- 32+ GB RAM
- 8+ CPU cores
- 50+ GB free disk space (depending on dataset size)

Tested Operating Systems:

- Linux (Ubuntu 20.04+, CentOS 7+)

Virtual Environment (Recommended)

Create and activate a conda environment:

```
conda create -n genophi python=3.10
conda activate genophi
```

Install GenoPHI

Clone and install from GitHub:

```
git clone https://github.com/Noonanav/GenoPHI.git
cd GenoPHI
pip install -e .
```

For development with optional dependencies:

```
pip install -e ".[dev]"
```

Verify Installation

Test that GenoPHI is properly installed:

```
# Check GenoPHI version
genophi --version

# Verify MMseqs2 is accessible
mmseqs version

# Run basic help command
genophi --help
```

Quick Start

GenoPHI provides a unified command-line interface accessible through the `genophi` command:

```
# View available commands
genophi --help

# Get help for a specific command
genophi protein-family-workflow --help
```

Recommended Default Run

For most phage-host interaction prediction tasks, use these recommended settings:

```
genophi protein-family-workflow \
    --input_strain strain_fastas/ \
    --input_phage phage_fastas/ \
    --phenotype_matrix interactions.csv \
    --output results/ \
    --threads 8 \
    --num_features 100 \
    --num_runs_fs 25 \
    --num_runs_modeling 50 \
    --method rfe \
    --use_clustering \
    --cluster_method hierarchical \
    --n_clusters 20 \
    --filter_type strain
```

Key Parameters Explained:

- `--num_features 100`: Select top 100 features (adjust based on dataset size)
- `--num_runs_fs 25`: 25 iterations for robust feature selection
- `--num_runs_modeling 50`: 50 modeling runs for reliable performance estimates
- `--method rfe`: Recursive Feature Elimination (balanced performance)
- `--use_clustering`: Enable hierarchical clustering of features
- `--filter_type strain`: Split data by strain to test generalization

For single-strain phenotypes (no phage data):

```
genophi protein-family-workflow \
    --input_strain strain_fastas/ \
    --phenotype_matrix phenotypes.csv \
    --output results/ \
    --threads 8 \
    --sample_column strain \
    --phenotype_column resistance \
    --filter_type none
```

Usage

CLI Commands Overview

GenoPHI provides the following main commands:

Command	Description
`protein-family-workflow`	**Recommended basic workflow**: Complete protein family-based workflow
`full-workflow`	Protein families → *k*-mers from predictive proteins

Command	Description
`kmer-workflow`	Complete *k*-mer-based workflow from all proteins
`cluster`	Generate protein family clusters and feature tables
`select-features`	Perform feature selection on any feature table
`train`	Train predictive models on selected features
`predict`	Predict phenotypes using trained models
`select-and-train`	Feature selection + modeling from any feature table
`assign-features`	Assign features to new genomes
`assign-predict`	Assign features and predict (protein families)
`annotate`	Annotate predictive features with functional info
`kmer-assign-features`	Assign *k*-mer features to new genomes
`kmer-assign-predict`	Assign *k*-mer features and predict

Workflows

1. Protein Family Workflow (Recommended)

The primary workflow for most applications. Performs complete protein family clustering, feature selection, and modeling.

Complete Workflow

```
genophi protein-family-workflow \
    --input_strain strain_fastas/ \
    --input_phage phage_fastas/ \
    --phenotype_matrix interactions.csv \
    --output results/ \
    --threads 8 \
    --num_features 100 \
    --num_runs_fs 25 \
    --num_runs_modeling 50 \
    --method rfe \
    --filter_type strain
```

Output Structure:

```
results/
├── clustering/              # MMseqs2 databases and clusters
├── feature_tables/          # Generated feature tables
├── feature_selection/       # Selected features and occurrence counts
├── modeling_results/        # Models and performance metrics
```

```
│   ├── cutoff_5/, cutoff_10/, ...
│   ├── model_performance/   # Summary plots
│   └── models/              # Trained models for prediction
└── workflow_report.txt      # Performance and timing summary
```

Single-Strain Phenotype Prediction

For strain-level phenotypes (no phage data required):

```
genophi protein-family-workflow \
    --input_strain strain_fastas/ \
    --phenotype_matrix strain_phenotypes.csv \
    --output results/ \
    --threads 8 \
    --sample_column strain \
    --phenotype_column antibiotic_resistance \
    --task_type classification \
    --filter_type none
```

Phenotype Matrix Format:

```
strain,antibiotic_resistance
Strain_001,1
Strain_002,0
Strain_003,1
```

For regression:

```
--task_type regression \
--phenotype_column growth_rate
```

2. Full Workflow: Protein Families → Predictive *K*-mers

This workflow first identifies predictive protein families, then extracts *k*-mers specifically from those families for refined modeling. This combines the interpretability of protein families with the resolution of *k*-mer analysis.

```
genophi full-workflow \
    --input_strain strain_fastas/ \
    --input_phage phage_fastas/ \
    --phenotype_matrix interactions.csv \
    --output results/ \
    --k 5 \
    --threads 8
```

Workflow Steps:

1. Cluster proteins into families
2. Perform feature selection on protein families
3. Extract *k*-mers from predictive protein families only
4. Train models on *k*-mer features
5. Generate annotations for predictive *k*-mers

3. *K*-mer Workflow (*De Novo*)

Generate *k*-mer features from all proteins without prior protein family analysis:

```
genophi kmer-workflow \
    --input_strain strain_fastas/ \
    --input_phage phage_fastas/ \
    --phenotype_matrix interactions.csv \
    --output kmer_results/ \
    --k 4 \
    --threads 8 \
    --num_features 100 \
    --num_runs_fs 25 \
    --num_runs_modeling 50
```

***K*-mer Specific Parameters:**

- `--k 4`: *K*-mer length (default: 4)
- `--k_range`: Generate *k*-mers from length 3 to k
- `--one_gene`: Include features with only one gene (default: False)

Advanced Options:

```
--use_dynamic_weights \        # Apply dynamic weighting
--weights_method inverse_frequency \       # Weighting method
--use_feature_clustering \     # Pre-filter by cluster presence
--feature_n_clusters 20        # Number of feature clusters
```

4. Modular Step-by-Step Workflows

Step 1: Clustering and Feature Generation

```
genophi cluster \
    --input_strain strain_fastas/ \
    --input_phage phage_fastas/ \
    --phenotype_matrix interactions.csv \
    --output clustering_results/ \
    --min_seq_id 0.4 \
```

```
    --coverage 0.8 \
    --sensitivity 7.5 \
    --threads 8
```

Clustering Parameters:

- `--min_seq_id 0.4`: Minimum sequence identity (range: 0-1)
- `--coverage 0.8`: Minimum coverage (range: 0-1)
- `--sensitivity 7.5`: MMseqs2 sensitivity (higher = more sensitive, slower)

Step 2: Feature Selection

Feature selection works on **any** feature table with a phenotype column:

```
genophi select-features \
    --input feature_table.csv \
    --output feature_selection/ \
    --method rfe \
    --num_features 100 \
    --num_runs 25 \
    --filter_type strain \
    --phenotype_column interaction \
    --threads 8
```

Feature Selection Methods:

- `rfe`: Recursive Feature Elimination (recommended)
- `shap_rfe`: RFE using SHAP values
- `select_k_best`: ANOVA F-test (fast)
- `chi_squared`: Chi-squared test
- `lasso`: L1 regularization
- `shap`: Direct SHAP importance

Advanced Selection Options:

```
--use_dynamic_weights \              # Handle imbalanced features
--weights_method inverse_frequency \  # Weighting strategy
--use_clustering \                   # Group correlated features
--cluster_method hierarchical \      # Clustering algorithm
--n_clusters 20                      # Number of clusters
```

Step 3: Model Training

Train models from selected features or any feature table:

```
genophi train \
    --input_dir feature_selection/filtered_feature_tables \
    --output models/ \
    --num_runs 50 \
    --phenotype_column interaction \
    --threads 8
```

For regression tasks:

```
--task_type regression \
--phenotype_column efficiency
```

Step 4: Select-and-Train (Combined)

Run feature selection and modeling together from **any feature table**:

```
genophi select-and-train \
    --input custom_feature_table.csv \
    --output results/ \
    --method rfe \
    --num_features 100 \
    --num_runs_fs 25 \
    --num_runs_modeling 50 \
    --phenotype_column your_phenotype \
    --sample_column your_sample_id \
    --threads 8
```

This command is flexible and works with:

- Protein family features
- *K*-mer features
- Custom features
- Any feature table with any phenotype / output column

5. Prediction Workflows

Assign Features and Predict (Protein Families)

```
genophi assign-predict \
    --input_dir new_strains/ \
    --mmseqs_db results/tmp/strain/mmseqs_db \
    --clusters_tsv results/strain/clusters.tsv \
    --feature_map results/strain/features/selected_features.csv \
    --model_dir results/modeling_results/cutoff_* \
    --phage_feature_table results/phage/features/feature_table.csv \
```

```
    --output predictions/ \
    --genome_type strain
```

For new phages:

```
--input_dir new_phages/ \
--mmseqs_db results/tmp/phage/mmseqs_db \
--clusters_tsv results/phage/features/selected_features.csv \
--strain_feature_table results/strain/features/feature_table.csv \
--genome_type phage
```

_K_-mer Assignment and Prediction

```
genophi kmer-assign-predict \
    --input_dir new_strains/ \
    --mmseqs_db results/tmp/strain/mmseqs_db \
    --clusters_tsv results/strain/clusters.tsv \
    --feature_map results/strain/features/selected_features.csv \
    --filtered_kmers kmer_results/kmer_tables/filtered_kmers.csv \
    --aa_sequence_file kmer_results/kmer_tables/aa_sequences.faa \
    --model_dir kmer_results/modeling/modeling_results/cutoff_* \
    --output predictions/ \
    --genome_type strain
```

6. Feature Annotation

Identifies proteins associated predictive protein families or *k*-mers and merges with functional information:

```
genophi annotate \
    --selected_features
feature_selection/filtered_feature_tables/select_feature_table_cutoff_3.csv \
    --feature_map strain/features/feature_map.csv \
    --clusters_tsv clustering_results/clustering/selected_features.csv \
    --annotation_table annotations.csv \
    --aa_sequence_file all_sequences.faa \
    --output annotations/ \
    --feature_type strain
```

Input Data Formats

FASTA Files

Protein sequences in FASTA format (.faa files):

```
>protein_id_1
MKTAYIAKQRQISFVKSHFSRQLEERLGLIEVQAPILSRVGDGTQDNLSGAEKAVQVKVKALPDAQFEVVHSLAKWKRQ...
>protein_id_2
MRISTTITTTITITTGNGAG...
```

Important: Protein IDs must be unique across all genomes. If duplicates exist, GenoPHI will automatically prefix them with genome names.

Phenotype Matrix

Phage-Host Interactions

Binary classification (infection/no infection):

```
strain,phage,interaction
Strain_001,Phage_A,1
Strain_001,Phage_B,0
Strain_002,Phage_A,1
```

Regression (infection efficiency):

```
strain,phage,efficiency
Strain_001,Phage_A,0.85
Strain_001,Phage_B,0.12
Strain_002,Phage_A,0.93
```

Single-Strain Phenotypes

Classification:

```
strain,antibiotic_resistance
Strain_001,1
Strain_002,0
Strain_003,1
```

Regression:

```
strain,growth_rate
Strain_001,0.42
Strain_002,0.38
Strain_003,0.51
```

Column Names: Use `--strain_column`, `--phage_column`, `--sample_column`, and `--phenotype_column` to specify your column names.

Feature Selection Methods

Method	Description	Best For	Speed
RFE (recommended)	Recursive Feature Elimination	General use, balanced performance	Medium
SHAP-RFE	RFE using SHAP values	Model-agnostic importance	Slow (High RAM)
SelectKBest	ANOVA F-test	Fast screening, linear relationships	Fast
Chi-squared	χ^2 test for independence	Categorical features	Fast
Lasso	L1 regularized regression	Sparse models, multicollinearity	Fast
SHAP	Shapley Additive Explanations	Direct feature importance	Slow (High RAM)

Dynamic Weighting

Handle imbalanced feature distributions:

```
--use_dynamic_weights \
--weights_method inverse_frequency  # or log10, balanced
```

When to use:

- Features with highly variable occurrence frequencies
- Some features present in most genomes, others very rare
- Imbalanced positive/negative examples

Clustering-Based Selection

Group correlated features for more robust selection:

```
--use_clustering \
--cluster_method hierarchical \  # or hdbscan
--n_clusters 20
```

HDBSCAN Options:

```
--cluster_method hdbscan \
--min_cluster_size 5 \
```

```
  --min_samples 5 \
  --cluster_selection_epsilon 0.0
```

Performance Metrics

Classification Metrics

- **AUC-ROC (Area Under ROC Curve)**: Overall discriminative ability $$\text{AUC} = \int_{0}^{1} \text{TPR} (\text{FPR}) , d\text{FPR}$$
- **MCC (Matthews Correlation Coefficient)**: Balanced metric for all confusion matrix elements $$\text{MCC} = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}$$
- **Precision**: Proportion of true positives among predicted positives $$\text{Precision} = \frac{TP}{TP + FP}$$
- **Recall (Sensitivity)**: Proportion of true positives among actual positives $$\text{Recall} = \frac{TP}{TP + FN}$$
- **F1 Score**: Harmonic mean of precision and recall $$F1 = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}}$$
- **Accuracy**: Overall correct predictions $$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}$$

Regression Metrics

- **RMSE**: Root Mean Squared Error
- **MAE**: Mean Absolute Error
- **R^2**: Coefficient of determination

Visualization Outputs

GenoPHI generates comprehensive visualizations:

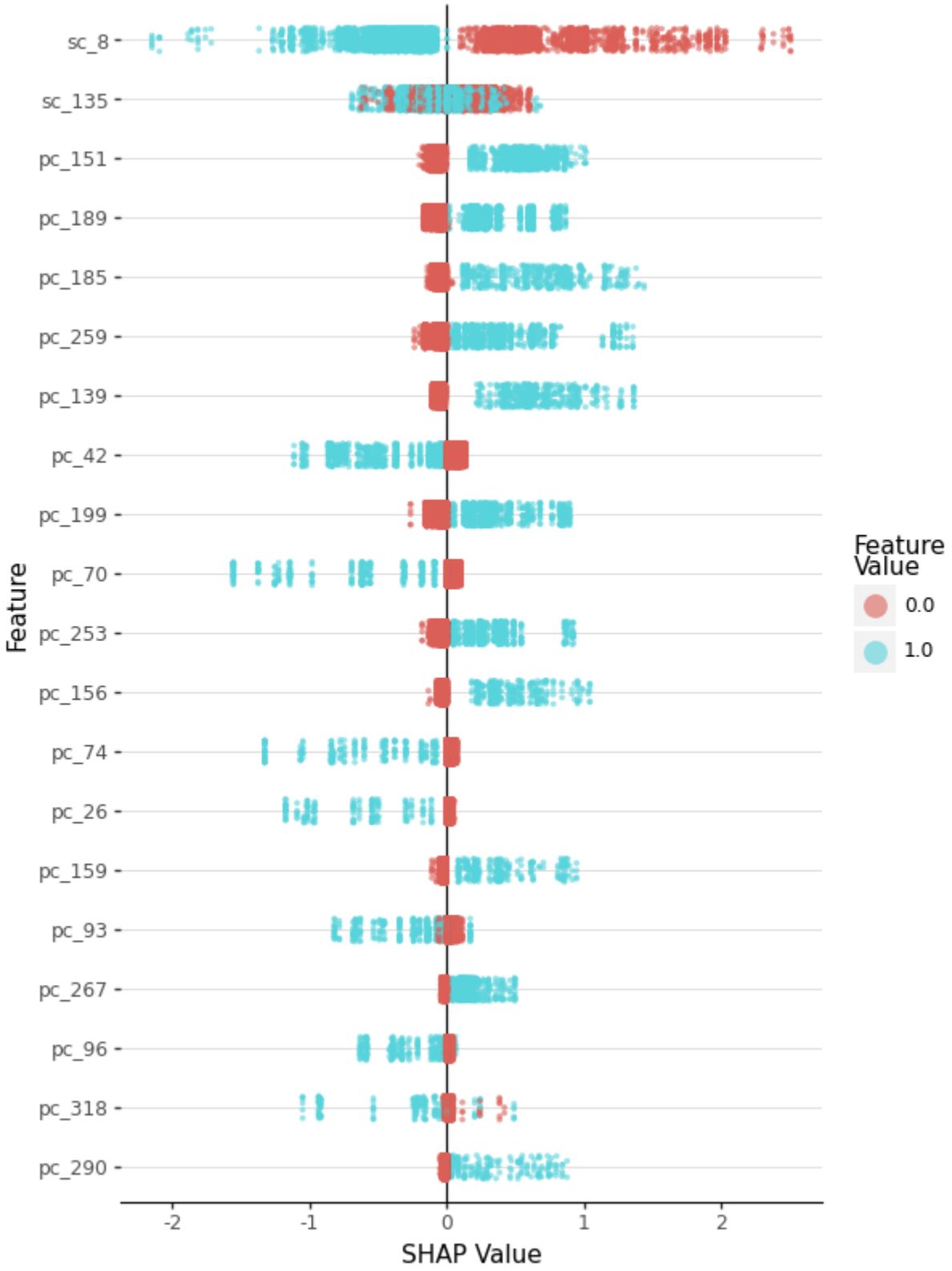
Per-Run Plots (`modeling_results/cutoff_*/run_*/`):

- Confusion matrices (classification)
- ROC curves with AUC scores
- Precision-Recall curves
- SHAP feature importance bar plots
- SHAP value scatter plots (beeswarm)

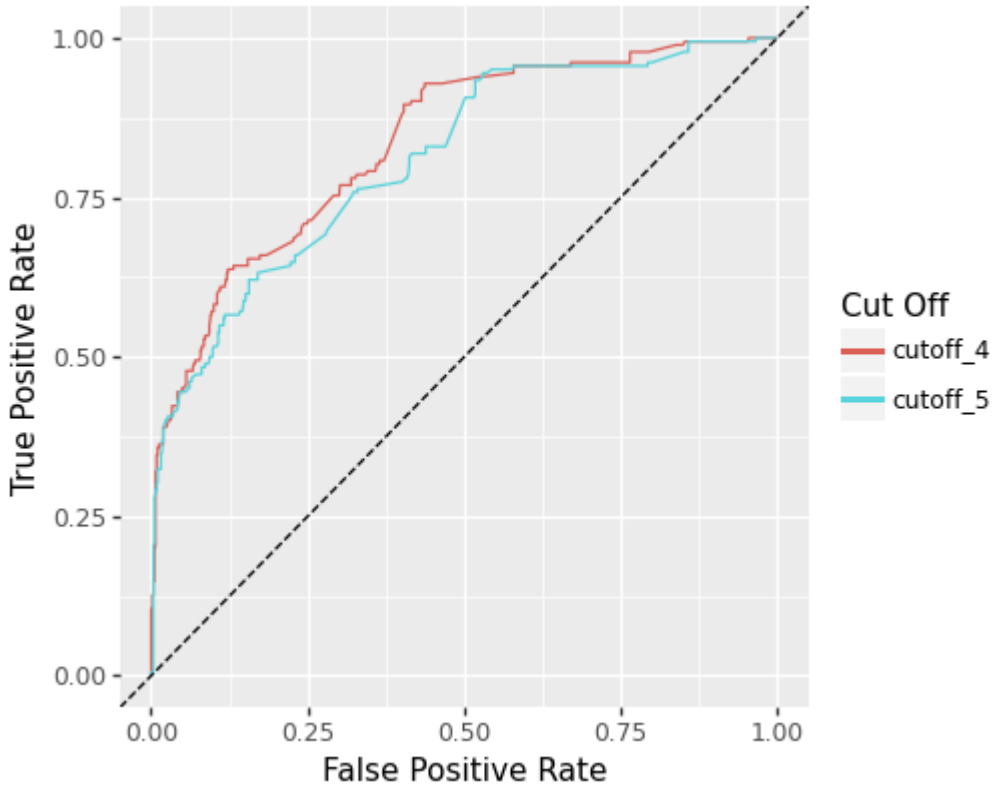
Summary Plots (`modeling_results/model_performance/`):

- SHAP beeswarm plots across all runs
- ROC curve comparisons across feature selection cutoffs
- Precision-Recall curve comparisons
- Hit rate and hit ratio curves

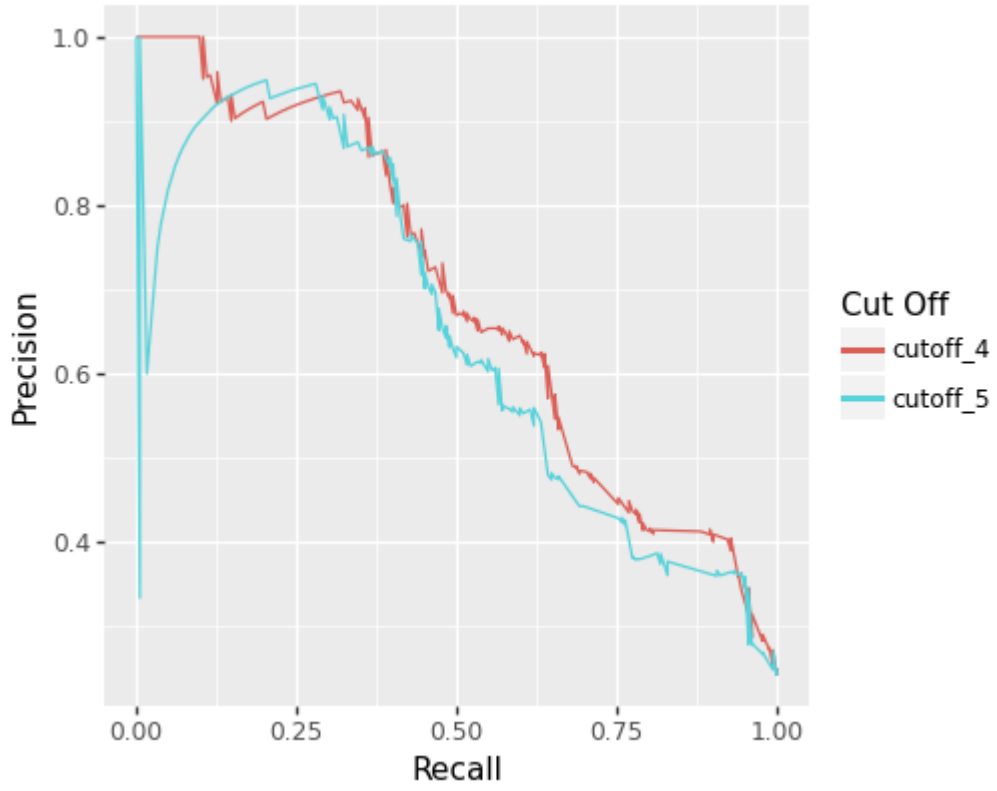
Example visualizations from the original README:



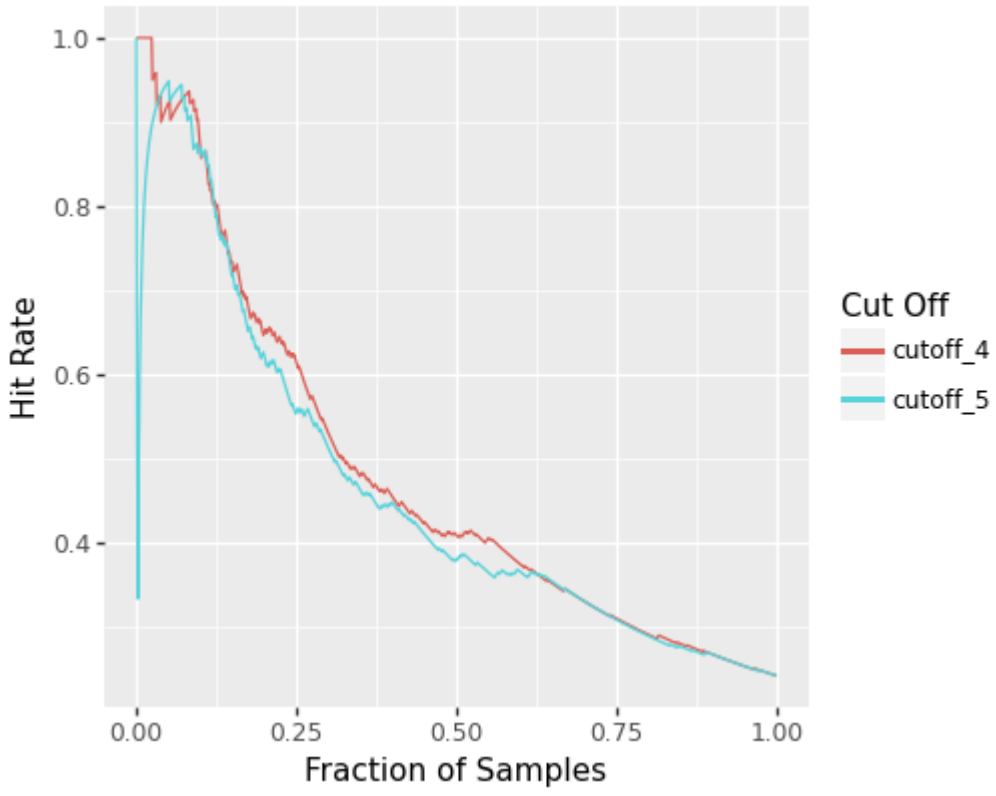
SHAP feature importance summary



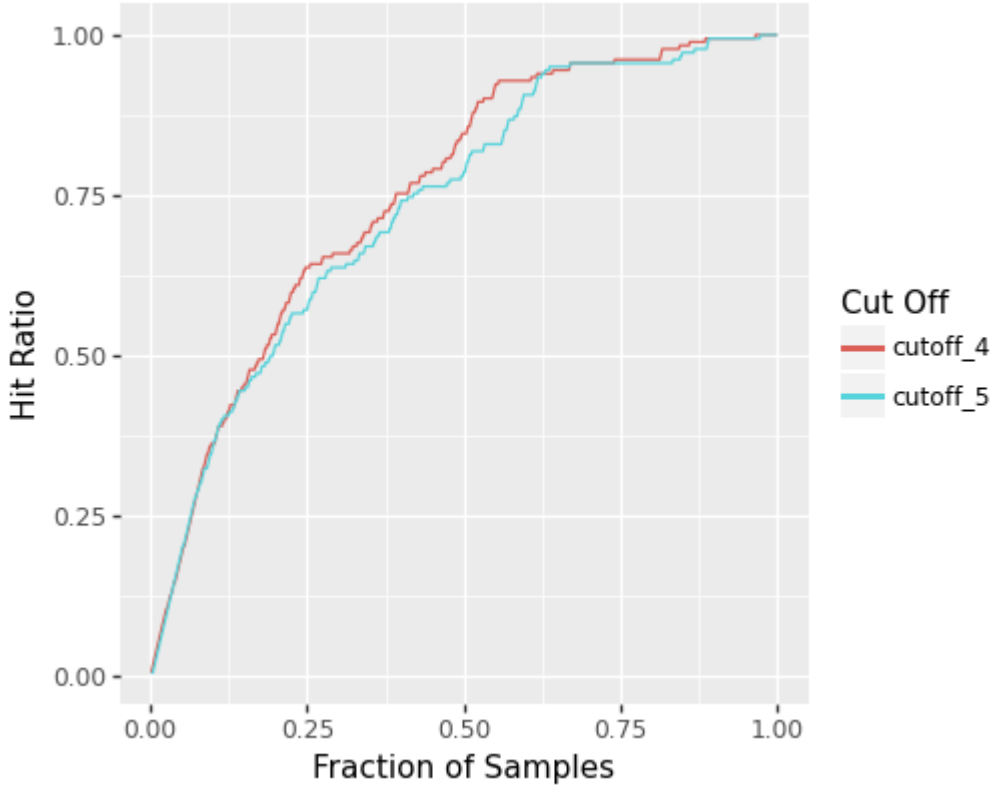
ROC curves comparing different feature selection cutoffs



Precision-Recall curves



Hit rate analysis



Hit ratio analysis

Output Directory Structure

Protein Family Workflow

```
output_dir/
├── clustering/
│   ├── strain_db/              # MMseqs2 database
│   ├── strain_clusters.tsv     # Cluster assignments
```

```
│   ├── phage_db/               # Phage database (optional)
│   └── phage_clusters.tsv
├── feature_tables/
│   ├── strain_feature_table.csv
│   ├── phage_feature_table.csv  # If phages provided
│   ├── merged_feature_table.csv
│   └── feature_map.csv
├── feature_selection/
│   ├── selected_features/
│   │   ├── run_1_selected_features.csv
│   │   └── ...
│   ├── feature_occurrence_counts.csv
│   └── filtered_feature_tables/
│       ├── cutoff_5_feature_table.csv
│       ├── cutoff_10_feature_table.csv
│       └── ...
├── modeling_results/
│   ├── cutoff_5/
│   │   ├── run_1/, run_2/, ...
│   │   └── cutoff_5_combined_performance.csv
│   ├── cutoff_10/
│   ├── model_performance/
│   │   ├── shap_summary_plots/
│   │   ├── roc_curve.png
│   │   ├── pr_curve.png
│   │   └── performance_comparison.csv
│   └── models/
│       ├── run_1/best_model.pkl
│       └── ...
├── predictions/
│   ├── predicted_interactions.csv
│   └── prediction_confidence.csv
└── workflow_report.txt
```

K-mer Workflow

```
output_dir/
├── clustering/                 # Protein family clustering
├── kmer_tables/
│   ├── kmer_feature_table.csv
│   ├── feature_map.csv
│   ├── filtered_kmers.csv
│   ├── feature2cluster.csv
│   └── aa_sequences.faa
├── feature_selection/
├── modeling_results/
└── kmer_workflow_report.txt
```

Python API

GenoPHI can also be used programmatically:

```
from genophi.workflows import (
    run_protein_family_workflow,
    run_kmer_workflow,
    run_modeling_workflow_from_feature_table,
    assign_predict_workflow
)

# Recommended: Protein family workflow
run_protein_family_workflow(
    input_path_strain="strain_fastas/",
    input_path_phage="phage_fastas/",
    phenotype_matrix="interactions.csv",
    output_dir="results/",
    threads=8,
    num_features=100,
    num_runs_fs=50,
    num_runs_modeling=100,
    method='rfe',
    filter_type='strain'
)

# _K_-mer workflow
run_kmer_workflow(
    input_strain_dir="strain_fastas/",
    input_phage_dir="phage_fastas/",
    phenotype_matrix="interactions.csv",
    output_dir="kmer_results/",
    k=5,
    threads=8,
    num_features=100
)

# Feature selection and modeling from any feature table
run_modeling_workflow_from_feature_table(
    full_feature_table="custom_features.csv",
    output_dir="modeling_results/",
    phenotype_column="your_phenotype",
    sample_column="your_sample_id",
    num_features=100,
    num_runs_fs=50,
    num_runs_modeling=100,
    method='rfe'
)

# Prediction workflow
assign_predict_workflow(
    input_dir="new_genomes/",
    mmseqs_db="results/tmp/strain/mmseqs_db",
    clusters_tsv="results/strain/clusters.tsv",
    feature_map="results/strain/features/selected_features.csv",
    model_dir="results/modeling_results/cutoff_*",
```

```
    output_dir="predictions/",
    genome_type='strain',
    phage_feature_table_path="results/phage/features/feature_table.csv"
)
```

Advanced Usage

Custom Train-Test Splits

Control how data is split for model evaluation:

```
--filter_type none      # Random split
--filter_type strain    # Leave-strain-out (test on new strains)
--filter_type phage     # Leave-phage-out (test on new phages)
```

Recommendation: Use `--filter_type strain` for phage-host predictions to evaluate generalization to new strains.

Hyperparameter Tuning

Models use grid search for hyperparameter optimization. Default parameters are optimized for phage-host interaction prediction but can be customized in the code.

Handling Large Datasets

For large datasets, adjust memory and threading:

```
--max_ram 64 \          # Maximum RAM in GB
--threads 16 \          # Number of CPU threads
--clear_tmp             # Remove temporary files after completion
```

Regression Tasks

For continuous phenotypes:

```
--task_type regression \
--phenotype_column efficiency
```

GenoPHI will use appropriate regression metrics (RMSE, MAE, R^2) instead of classification metrics.

Pre-processing Feature Clustering

Filter features by cluster presence before modeling:

```
--use_feature_clustering \
--feature_cluster_method hierarchical \
--feature_n_clusters 20 \
--feature_min_cluster_presence 2
```

This removes features that appear in fewer than `feature_min_cluster_presence` genome clusters.

Example Datasets

To help you get started, we recommend testing GenoPHI with:

Test Dataset Structure

Minimal test case with 10 strains, 5 phages:

```
test_data/
├── strains/
│   ├── Strain_001.faa
│   ├── Strain_002.faa
│   └── ...
├── phages/
│   ├── Phage_A.faa
│   ├── Phage_B.faa
│   └── ...
└── interactions.csv
```

Troubleshooting

Common Issues

Issue: MMseqs2 not found

```
Solution: Ensure MMseqs2 is installed and in your PATH
conda install -c bioconda mmseqs2
which mmseqs  # Should show the path
```

Issue: Out of memory errors

```
Solution:
- Reduce --max_ram parameter
- Process fewer genomes at once
- Use --clear_tmp to remove intermediate files
- Increase system swap space
```

Issue: Duplicate protein IDs

```
Solution: GenoPHI automatically detects and prefixes duplicates with genome names
If you want to prevent this, ensure protein IDs are unique across all input files
```

Issue: No predictive features found

```
Solution:
- Try different feature selection methods (--method)
- Adjust num_features parameter
- Check that phenotype matrix has sufficient positive/negative examples
- Verify that interaction matrix matches genome filenames
```

Issue: Poor model performance

```
Solution:
- Increase num_runs_fs and num_runs_modeling for more robust results
- Try different feature selection methods
- Use --use_dynamic_weights for imbalanced features
- Enable --use_clustering for feature grouping
- Check data quality and ensure phenotype matrix is correct
- Try different clustering parameters (min_seq_id, coverage)
```

Issue: Models take too long to train

```
Solution:
- Reduce num_runs_modeling
- Reduce num_features
- Increase --threads parameter
- Use faster feature selection methods (select_k_best, chi_squared)
```

Frequently Asked Questions (FAQ)

General Questions

Q: Can GenoPHI be used for organisms other than phages and bacteria?
A: Yes! While designed for phage-host interactions, GenoPHI works with any protein sequences and phenotypes. It's been applied to bacteria, archaea, and eukaryotic microbes.

Q: How many genomes do I need for reliable predictions?
A: Minimum: ~20 strains and 20 phages with ~400 interactions. Recommended: 50+ strains, 50+ phages, 5000+ interactions for robust models.

Q: What file formats are required?
A: FASTA files (.faa) for protein sequences and CSV for phenotype matrices. See Input Data Formats for details.

Technical Questions

Q: What's the difference between protein family and *k*-mer approaches?
A: Protein families group similar full-length proteins (interpretable, captures protein-level patterns). *K*-mers analyze short amino acid sequences (high resolution, captures local patterns). The `full-workflow` combines both by extracting *k*-mers from predictive protein families.

Q: Should I use single-strain or phage-host mode?
A: Use phage-host mode for interaction prediction. Use single-strain mode for strain-level phenotypes (resistance, growth rate, etc.) where phage data isn't relevant.

Q: Which feature selection method should I use?
A: Start with RFE (balanced performance). Try SHAP for interpretability or SelectKBest for speed. Compare multiple methods for best results.

Q: How do I interpret SHAP plots?
A: SHAP beeswarm plots show feature importance. Features at the top are most important. Red dots = high feature values, blue = low. Position right of center = positive impact on prediction.

Q: Can I use custom features instead of protein families?
A: Yes! Use `select-and-train` with any feature table containing a phenotype column (metabolic pathways, gene presence/absence, etc.).

Q: How do I handle imbalanced datasets?
A: Use `--use_dynamic_weights` with `--weights_method inverse_frequency` to balance feature importance. CatBoost also has built-in class balancing.

Troubleshooting Questions

Q: Models perform poorly - what should I try?
A: (1) Increase num_runs for more robust estimates, (2) Try different clustering parameters, (3) Enable dynamic weighting, (4) Check data quality and phenotype matrix accuracy.

Q: How much RAM do I need?
A: Minimum 8 GB. Recommend 16+ GB for 50+ genomes, 32+ GB for 100+ genomes. Use `--max_ram` to limit memory usage.

Best Practices

1. **Start with recommended defaults** for initial analysis
2. **Use `--filter_type strain`** for phage-host predictions to evaluate generalization
3. **Run multiple iterations** (`num_runs_fs = 25`, `num_runs_modeling = 50`) for robust results
4. **Enable clustering** (`--use_clustering`) for correlated features
5. **Check data quality** before modeling - ensure phenotype matrix matches genome filenames
6. **Use SHAP plots** to understand which features drive predictions
7. **Compare multiple feature selection methods** to find optimal features
8. **For large datasets**, start with a subset to optimize parameters

Version History

v0.1.0 (Current)

- Initial release
- Protein family-based workflow with MMseqs2 clustering
- *K*-mer-based workflow with flexible *k*-mer lengths
- Multiple feature selection methods (RFE, SHAP, SelectKBest, Chi-squared, Lasso)
- CatBoost model training with hyperparameter optimization
- SHAP-based interpretability
- Support for classification and regression tasks
- Single-strain and phage-host prediction modes
- Unified CLI with 12 commands
- Comprehensive visualization outputs

Upcoming Features

- Web interface for prediction and visualization
- Docker container for easy deployment

Publication Datasets

The datasets used in the GenoPHI publication are included in the `data/` directory for reproducibility and benchmarking purposes.

```
data/
├── experimental_validation/
│   ├── BASEL_ECOR_interaction_matrix.csv    # BASEL collection against ECOR
strains for model validation
│   └── ECOR27_TnSeq_high_fitness_genes.csv  # Filtered RB-TnSeq results
└── interaction_matrices/
    ├── ecoli_interaction_matrix.csv          # E. coli phage-host interactions
    ├── ecoli_interaction_matrix_subset.csv   # Smaller E. coli subset for testing
    ├── klebsiella1_interaction_matrix.csv    # Klebsiella dataset 1
    ├── klebsiella2_interaction_matrix.csv    # Klebsiella dataset 2
    ├── pseudomonas_interaction_matrix.csv    # Pseudomonas interactions
    └── vibrio_interaction_matrix.csv         # Vibrionaceae interactions
```

Citation

If you use GenoPHI in your research, please cite:

```
@article{noonan2025genophi,
  author = {Noonan, Avery J. C. and Moriniere, Lucas and Rivera-López, Edwin O.
and Patel, Krish and Pena, Melina and Svab, Madeline and Kazakov, Alexey and
Deutschbauer, Adam and Dudley, Edward G. and Mutalik, Vivek K. and Arkin, Adam
P.},
  title = {Phylogeny-agnostic strain-level prediction of phage-host interactions
from genomes},
  year = {2025},
```

```
    doi = {10.1101/2025.11.15.688630},
    publisher = {Cold Spring Harbor Laboratory},
    url = {https://www.biorxiv.org/content/10.1101/2025.11.15.688630v1},
    journal = {bioRxiv}
  }
```

Preprint: Noonan, A.J.C., Moriniere, L., Rivera-López, E.O., Patel, K., Pena, M., Svab, M., Kazakov, A., Deutschbauer, A., Dudley, E.G., Mutalik, V.K., & Arkin, A.P. (2025). Phylogeny-agnostic strain-level prediction of phage-host interactions from genomes. *bioRxiv*. https://doi.org/10.1101/2025.11.15.688630

Contributing

We welcome contributions to GenoPHI! Here's how you can help:

Ways to Contribute

- **Report bugs** or suggest features via GitHub Issues
- **Submit pull requests** for bug fixes or new features
- **Improve documentation** by fixing typos or adding examples
- **Share your use cases** and publications using GenoPHI
- **Test on different platforms** and report compatibility issues

Development Guidelines

1. Fork the repository and create a feature branch
2. Follow PEP 8 style guidelines for Python code
3. Add tests for new functionality
4. Update documentation as needed
5. Submit a pull request with a clear description of changes

Code of Conduct

We are committed to providing a welcoming and inclusive environment. Please be respectful and constructive in all interactions.

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Support

For questions, issues, or feature requests:

- Open an issue on GitHub
- Contact: Avery Noonan (averynoonan@gmail.com)

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