pyRforest: A comprehensive R package for genomic data analysis featuring scikit-learn Random Forests in R

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Abstract

Random Forest models are widely used in genomic data analysis and can offer insights into complex biological mechanisms, particularly where features influence the target in interactive, non-linear, or non-additive ways. Currently, some of the most efficient random forest methods, in terms of computational speed, are implemented in Python. However, many biologists use R for genomic data analysis, as R offers a unified platform for performing additional statistical analysis and visualization. Here we present an R package, *pyRforest*, which integrates Python *scikit-learn* `RandomForestClassifier` algorithms into the R environment. *pyRforest* inherits the efficient memory management and parallelization of Python, and is optimized for classification tasks on large genomic datasets, such as those from RNA-seq. *pyRforest* offers several additional capabilities, including a novel rank-based permutation method for biomarker identification. This method can be used to estimate and visualize p-values for individual features, allowing the researcher to identify a subset of features for which there is robust, statistical evidence of an effect. In addition, *pyRforest* includes methods for the calculation and visualization of SHapley ADditive Explanations (SHAP) values. Finally, *pyRforest* includes support for comprehensive downstream analysis for gene ontology and pathway enrichment. *pyRforest* thus improves the implementation and interpretability of random forest models for genomic data analysis by merging the strengths of Python with R. *pyRforest* can be downloaded at: https://www.github.com/tkolisnik/pyRforest with an associated vignette at https://github.com/tkolisnik/pyRforest/blob/main/vignettes/pyRforest-vignette.pdf.

Key Words: Bioinformatics, Machine Learning, Biomarker Identification, Random Forest, Genomic Data Analysis

Introduction

The field of genomics has evolved over the past two decades, from studies primarily focused on individual genes to comprehensive analyses of entire genomes [1]. This shift has been facilitated by advances in sequencing technologies and bioinformatics tools, allowing researchers to generate and analyze vast quantities of genomic data [2]. To derive meaningful biological insights from these large datasets, advanced statistical methods, such as machine learning, are increasingly required.

Decision trees are a supervised learning method frequently used for classification. The values of different features are used to split data along branches to make classification predictions. Random Forest (RF) models are ensembles of random trees, i.e., decision trees where only a random subset of features are used at each split, known for their high predictive power and robustness to dataset noise for handling large datasets, such as those of genomic research [3]. RFs can mitigate overfitting through mechanisms specifically designed to introduce variability among the individual trees, such as bootstrapping and feature bagging. These properties make RF models particularly competitive for binary classification problems [4].

Python [5] is often used for implementing RF models due to having libraries designed with efficient memory handling and parallelization in mind. However, R [6] is commonly used as a computing environment by biologists, and implementations of RF models in R are slow and computationally intensive compared to Python. Many RF implementations in R suffer from inefficient memory handling and lack parallelization strategies, which are crucial for processing large genomic datasets. This creates a barrier for researchers who must often choose between the advanced machine learning capabilities of Python and the comprehensive genomic analysis tools available in R.

To address these limitations, we present *pyRforest*, an R package that integrates the *scikit-learn* `RandomForestClassifier` algorithm [7] implemented in Python into the R environment using the *reticulate* package [8]. *pyRforest* enables users familiar with R to leverage the machine learning strengths of Python without requiring any Python coding knowledge. This integration improves memory management and parallel processing, allowing the user to create RF models on larger genomic datasets with less RAM, while enhancing the interpretability and biological relevance of RF models in genomic studies.

In addition, *pyRforest* offers several innovative features, including a novel rank-based permutation method for identifying significantly important features, which estimates and visualizes p-values for individual features. This allows researchers to prioritize a reduced list of biomarkers for further analysis, while speeding up computation. Additionally, *pyRforest* includes methods for calculating and visualizing SHapley ADditive Explanations (SHAP) values [9, 10] while supporting comprehensive downstream analysis for gene ontology and pathway enrichment with *clusterProfiler* [11] and *g:Profiler* [12]. By merging the computational strengths

of Python with the statistical and visualization capabilities of R, *pyRforest* addresses current limitations in genomic research workflows, contributing to the ongoing evolution towards more versatile and integrated bioinformatics tools and the need for more explainable artificial intelligence [13].

This paper introduces and outlines the capabilities of the *pyRforest* package for R. We first describe the development, use, and functionality of *pyRforest*. Next, we compare *pyRforest* to existing RF implementations, highlighting its computational advantages and innovative features. We then present a case study on colorectal cancer (CRC), demonstrating the utility of *pyRforest* in identifying key biomarkers and providing insights into their biological implications. Finally, we discuss the advantages and limitations of the package, as well as potential future directions for its development and application.

Methods and Design

R Package Development

pyRforest was created using the Rstudio IDE for R [14]. The *devtools* package [15] was used to streamline the code development, testing and documentation of the package. Github was used for version control and collaboration [16].

Integration of R and Python

The *pyRforest* setup process supports the built-in *virtualenv* environments of *reticulate* [8] as well as *Conda* [17] environments for Python package management. In our experience, *virtualenv* works best on Linux systems, and *Conda* is best for Apple Arm64/M series Mac users.

For detailed setup instructions, including a step-by-step guide for configuring *virtualenv* or *Conda* with *reticulate*, we refer users to a vignette included with *pyRforest*. The vignette includes a demonstration of the capabilities of *pyRforest* with an example dataset. The development of *pyRforest* and all case studies were carried out on an M1 iMac with 16GB RAM. The package was also tested successfully on Linux Ubuntu 20.04 LTS systems.

Dataset Preparation and Hyperparameter Optimization

pyRforest includes functions for formatting and partitioning data into training, validation, and testing sets. *pyRforest* optionally supports multiple class weighting strategies to ensure balance of the target variable across the data partitions, which can be particularly important if the sample size is unbalanced with respect to the target class.

pyRforest uses *scikit-learn* `GridSearchCV` [7] to optimize hyperparameters, with default settings that have been optimized using the genomic datasets presented here. However, *pyRforest*

allows users to customize hyperparameters, such as 'n_estimators' (number of decision trees), 'max_features' (maximum number of criteria for node splits), and 'max_depth' (maximum parent nodes on a decision tree), and others (see documentation for a full list) [7].

Model Tuning and Evaluation

The model is initially trained using the training set, and tuned using the validation set, with final assessment scores calculated on the testing set. The default metric for assessing model performance is the Area under the Receiver Operating Characteristic (ROC) curve (AUC) score, which is a combined measure of sensitivity and specificity. However, the user can choose from a range of other metrics, including precision and accuracy. The validation test set allows the user to test for model overfitting via the training set. For example, if training results in overfitting, such models will often underperform on the validation set; this poorer performance can be used as an indication of overfitting, requiring further tuning or pruning of hyperparameters.

After the hyperparameter-tuning phase, the testing set is used to assess model performance. Importantly, the testing set is not used in model fitting or in tests for overfitting. *pyRforest* provides the user with a range of scoring metrics, such as accuracy, ROC-AUC score, sensitivity, specificity, and F1 score.

Post-Hoc Feature Importance Significance Testing

pyRforest includes a procedure for identifying which features are significantly important to the predictive performance of the final model. The importance of each feature within the dataset is measured using the Gini importance score, and features are ranked from most to least important.

The statistical significance of each feature at each rank is determined using permutation to generate a null distribution of importance score profiles at each rank, to which the true importance scores of features at that rank are compared. This approach allows the calculation of p-values for each feature by comparing the observed importance score to the null distribution of importance scores obtained under permutation for the corresponding rank. By default, *pyRforest* performs 1,000 permutations (although this is customizable). This permutation test allows users to identify a subset of features for which there is statistical evidence of their importance.

Generating a Null Distribution of Importance Scores at Each Rank

The null distribution of importance scores for each rank is generated using the following steps:

1. Fit RF model; calculate importance scores for each feature; rank features from highest to lowest importance score

2. For each permutation (repeat 1000 times):

i. Randomly permute the values of the target variable across samples

ii. Fit RF model

iii. Calculate importance scores and rank them from highest to lowest

3. For each feature according to its importance rank:

i. Obtain the null distribution of importance scores obtained under permutation for that rank.

ii. Determine the p-value, the proportion of null importance scores that are greater than the observed importance score.

The p-values for each feature captures the probability of obtaining an importance score as great or greater than the one observed at its rank under a null hypothesis of no association between features and target variable.

In comparison, in the standard RF analysis, variable importance is assessed by the change in accuracy after the permutation of the value of a variable is obtained in an out-of-bag sample [3, 18]. Other permutation-based methods generally fall into two types: in one, class labels are swapped, and for each feature, the distribution of importance scores (frequently, Gini) is tested relative to the unpermuted dataset. For example, the *rfPermute* package [19] uses the mean decrease in Gini score for all permutations relative to the non-permuted data. In the other type, class labels are swapped, and for each feature, the distribution of the ranks is tested relative to the unpermuted dataset [20]. This contrasts to the case of *pyRforest*, in which the feature importance (Gini) at each rank is compared to the importance of the feature at that rank in the unpermuted dataset. More succinctly, *pyRforest* uses rank-based feature importance rather than feature-based importance, or feature-based *rank* importance.

The result is a list of features for which, given a chosen significance threshold (alpha), there is statistical evidence for non-zero importance to the predictive outcome of the final model. In a biological context, these features may be further studied as a list of potentially important biomarkers.

pyRforest also offers plotting functions to visualize these biomarkers, created with the popular R package *ggplot2* [21]. These plots can aid in the interpretation of the significance assessment. These permutation and visualization steps allow the researcher to determine which features (for example, genes) found by the RF model are deemed significant and worthy of further research.

SHAPley Additive exPlanations (SHAP)

SHAP values help explain the predictions of RF models, offering insight into when, why, and how specific features are important in determining class membership [9]. To facilitate this, *pyRforest* offers built-in functions for calculating and plotting SHAP values from the `shap.TreeExplainer` module of the *SHAP* package [10]. SHAP analysis can complement biological contextualization by providing a means to interpret the effects of individual features on model predictions.

Biological Interpretation

Finally, when using *pyRforest* on genomic data, *pyRforest* enhances biological interpretation of the produced ranked lists of significant genes, which are automatically prioritized and formatted for compatibility with downstream analytical tools. As shown in the examples within the vignette, the resultant data format is suitable for direct integration with *clusterProfiler* [11] and *g:Profiler* [12], powerful platforms for biological annotation and analysis. Specifically, these tools enable users to contextualize the statistically significant features reduced by *pyRforest* within biological pathways, functions, and processes.

Comparisons with alternative implementations

We first tested the computational advantages of the *reticulate*-based implementation of the Python *scikit-learn* `RandomForestClassifier` library in R. We compared the performance of *pyRforest* with two other RF packages implemented in R: *randomForest* [18] and *rfPermute* [19]. The reliance of *pyRforest* on the faster and more efficient RF functions in Python considerably sped up model fitting relative to the other packages (**Table 1**). Several other advantages of *pyRforest* relative to the two other RF implementations in R are shown in **Suppl. Table 1**. For example, in contrast to other tools, *pyRforest* supports direct class weighting, k-fold cross-validation, and simple integration with cross-validation tools, streamlining the analysis process.

Table 1. A comparative analysis assessing the number of features with non-zero feature importance as well as benchmarking, as observed in different feature identification approaches.

Method	**N Features with Non-Zero Importance**	**Run Time (hours)**	**Peak Memory Usage (16GB Max)**
Default *scikit-learn* `RandomForestClassifier`	1008	8 h	10GB
pyRforest	83	6 h	8GB
‘sklearn.inspection’ (1000 feature-based permutations)	1	95 h	16GB
randomForest R package	NA	NA	16GB

rfPermute R package	NA	NA	16GB

Our comparative analysis, as detailed in **Table 1**, demonstrates the nuanced capability of *pyRforest*. Of the 58,678 features available in our case study, *pyRforest* identified 83 genomic features (biomarkers) with significant importance. This is a considerably reduced set compared to the 1008 non-zero features that had non-zero importance in the default *scikit-learn* `RandomForestClassifier` model without any feature prioritization applied. The *pyRforest* approach offered a more robust method for feature identification compared to 'sklearn.inspection' which was extremely conservative, identifying only a single feature and missing several of the features known from other studies to affect CRC localization [22, 23]. Moreover, 'sklearn.inspection' had a significantly higher runtime than *pyRforest*. Additionally, *pyRforest* overcomes the memory handling inefficiencies that precluded use of the R packages *randomForest* and *rfPermute* on this large genomic large dataset.

In summary, *pyRforest* bridges the computational and methodological divide between Python and R, providing users with advantages of both: the advanced machine learning capabilities of Python *scikit-learn* and the comprehensive analysis strengths of R. It introduces an innovative rank-based permutation method tailored for biomarker prioritization in genomic data analysis. Additionally, pyRforest simplifies GO analysis with easy integration into *clusterProfiler* and *g:Profiler*. This unique combination of features positions *pyRforest* as a strong tool for end-to-end genomic studies.

Case Study

RNA-seq Data Analysis in Colorectal Cancer

In this case study, we employed *pyRforest* to analyze a dataset comprising RNA-seq data from colorectal cancer samples obtained from University of Otago, Christchurch, New Zealand analyzed in our previous study [22]. This dataset includes 308 patient samples, and encompasses a comprehensive range of 58,678 genomic features, including genes and long non-coding RNAs (lncRNAs). The primary focus of this analysis was to distinguish the cancer localization within the colorectum based on the binary outcome variable 'side' (left vs. right).

Data Preparation and Model Training

The raw genomic data was mapped to the human genome (GRCh38) using *STAR* (v2.73a) [24] and TPM (Transcripts Per Kilobase Million) normalized to remove sequencing depth and gene length biases. It was then split into training (248 samples), validation (30 samples), and testing (30 samples) sets, ensuring a robust evaluation for the RF model developed using *pyRforest*. The validation and testing sets were balanced for outcome, and the training set was minimally unbalanced with 57% left-sided samples and 43% right-sided samples. The RF model was trained and tuned using the *pyRforest* exhaustive grid search, yielding optimal hyperparameters

noted in **Supplementary Table 2**. The initial training of the model on the training set revealed 1008 features with non-zero Gini importance scores, indicating a significant level of complexity and feature interaction within the data **(Supplementary Table 3)**.

Table 2. K-fold cross validation RF model scoring metrics by dataset split on the CRC dataset from our case study.

Scoring Metric	Training Set	Validation Set	Testing Set
Accuracy	1.0	0.87	0.8
F1	1.0	0.88	0.8
Precision	1.0	0.78	0.8
Recall	1.0	1.0	0.8
ROC-AUC	1.0	0.88	0.8

Model Performance

In our case study, the RF model exhibited strong performance across a range of scoring metrics, including accuracy, F1 score, precision, recall and ROC-AUC score, as seen in **Table 2**. Notably, the model achieved high accuracy rates of 0.87 and 0.80 for the validation and testing sets respectively, with both F1 and ROC-AUC scores closely mirroring these values at 0.88 and 0.80. The model demonstrated strong generalization capabilities on the unseen testing dataset, and consistently high performance at discriminating between the outcome classes.

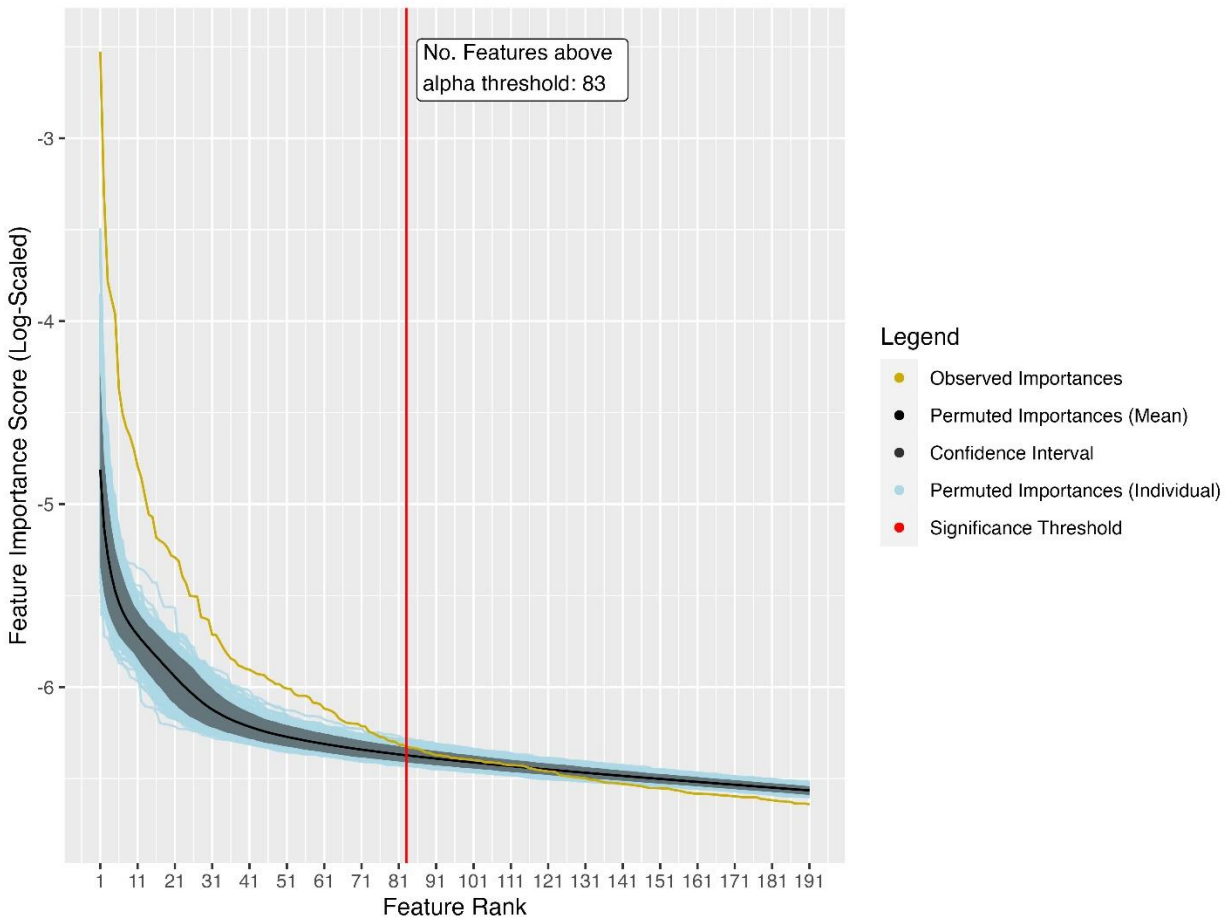


Figure 1. Feature importance plot showing individual and mean rank-based feature importance scores of the permuted data, with confidence intervals, and the scores of the observed data. The significance threshold was determined based on an alpha of 0.05, and is indicated by the red vertical line.

Identifying Significantly Important Features

The rank-based permutation approach of *pyRforest* identified 83 significantly important biomarkers of the total available set of 58,678 **(Figure 1).** Each feature was assigned a p-value, providing a statistical basis for their inclusion in the downstream analyses **(Supplementary Table 4).** Notably, the small nuclear protein *PRAC1* emerged as the top feature, indicating its potential role in the lateralization of CRC, which is a result consistent with many previous studies [22, 23]. *HOXB13*, and the lncRNA ENSG00000242407 were also identified as important features. *HOXB13* and this lncRNA colocalize with *PRAC1*: all are between 48.721 and 48.734 Mbp on chromosome 17. Their functional relationship to *PRAC1* is not clearcut. *HOXC4* and *HOXC6* were also identified; as developmental genes, these are functionally related to *HOXB13* and overlap on chromosome 12 between 53.991 and 54.017 Mbp. In addition, *QPRT* (tryptophan metabolism) and *WASF3* (cell movement and adhesion), were identified, both of known importance to CRC [25, 26]. Finally, *pyRforest* identified a second lncRNA,

ENSG0000250829 and this has no identified function, suggesting new avenues for future research. *PRAC1* was the sole feature identified by the `sklearn.inspection` feature identification approach**.**

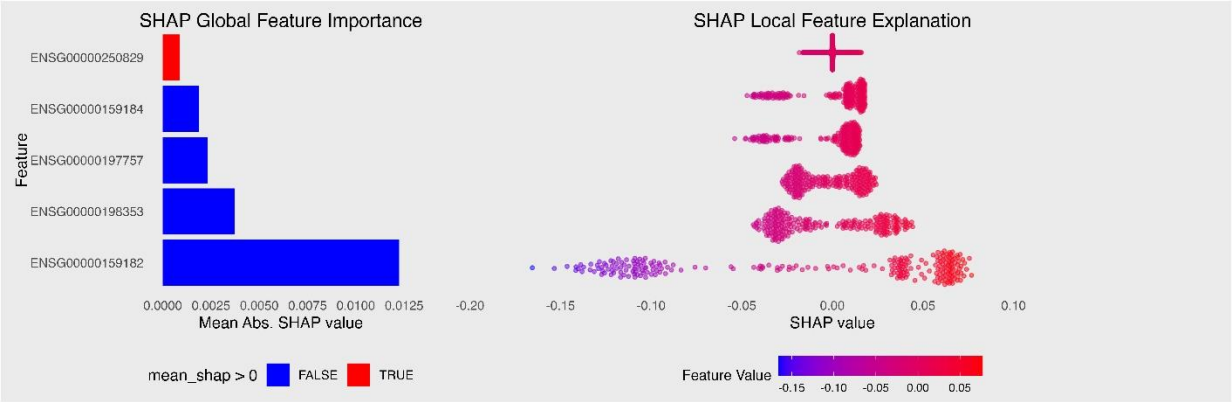


Figure 2. SHAPley Additive Explanation plots which illustrate the impact of individual features on model prediction.

The left panel displays the global feature importance with the mean absolute SHAP values, where blue signifies features driving predictions towards 'left-sided CRC' and red signifies features driving predictions towards 'right-sided CRC'.

The right panel shows the distribution of individual SHAP values for each feature, reflecting their contribution to each prediction - negative SHAP values suggest a push towards 'left-sided CRC', whereas positive values indicate a push towards 'right-sided CRC'. The color intensity corresponds to the feature value magnitude, with cooler colors representing lower values and warmer colors indicating higher values.

From top to bottom, the ENSEMBL Gene IDs in the figure above correspond to: the novel lncRNA transcript AC108865.1; HOXB13; HOXC6; HOXC4; and PRAC1.

SHAP Analysis

Calculation of SHAP values provides additional insights into the direction of the contribution (positive or negative) of these features to the target variable's outcome within the RF model, here defined as associated with left-sidedness **(Figure 2)**. SHAP importance rankings are independent from *pyRforest* rankings. Specifically, *SHAP* analysis indicated that *PRAC1* and *HOXB13* contribute to the predisposition towards left-sided CRC. This observation is consistent with outcomes from other studies, highlighting the role of these biomarkers as being differentially expressed depending on spatial location of the disease [22, 23]. *HOXC4* and *HOXC6* SHAP directionality deviated from expected values from our previous study: the bimodal feature explanations of these genes indicate the relationship of these genes with outcome may be

complex, and that their contribution is context dependent. In other words, depending on the expression level of other genes, these can contribute either positively or negatively toward left-sided CRC. SHAP analysis indicated that lncRNA ENSG0000250829 is the top feature contributing towards right-sided CRC, although the cross-shaped spread of the local feature explanation also indicates a complex, non-linear relationship with outcome. The inclusion of SHAP analysis enhances our understanding of the decisions of the predictive model, offering a clearer picture of how individual features can influence the output of the model.

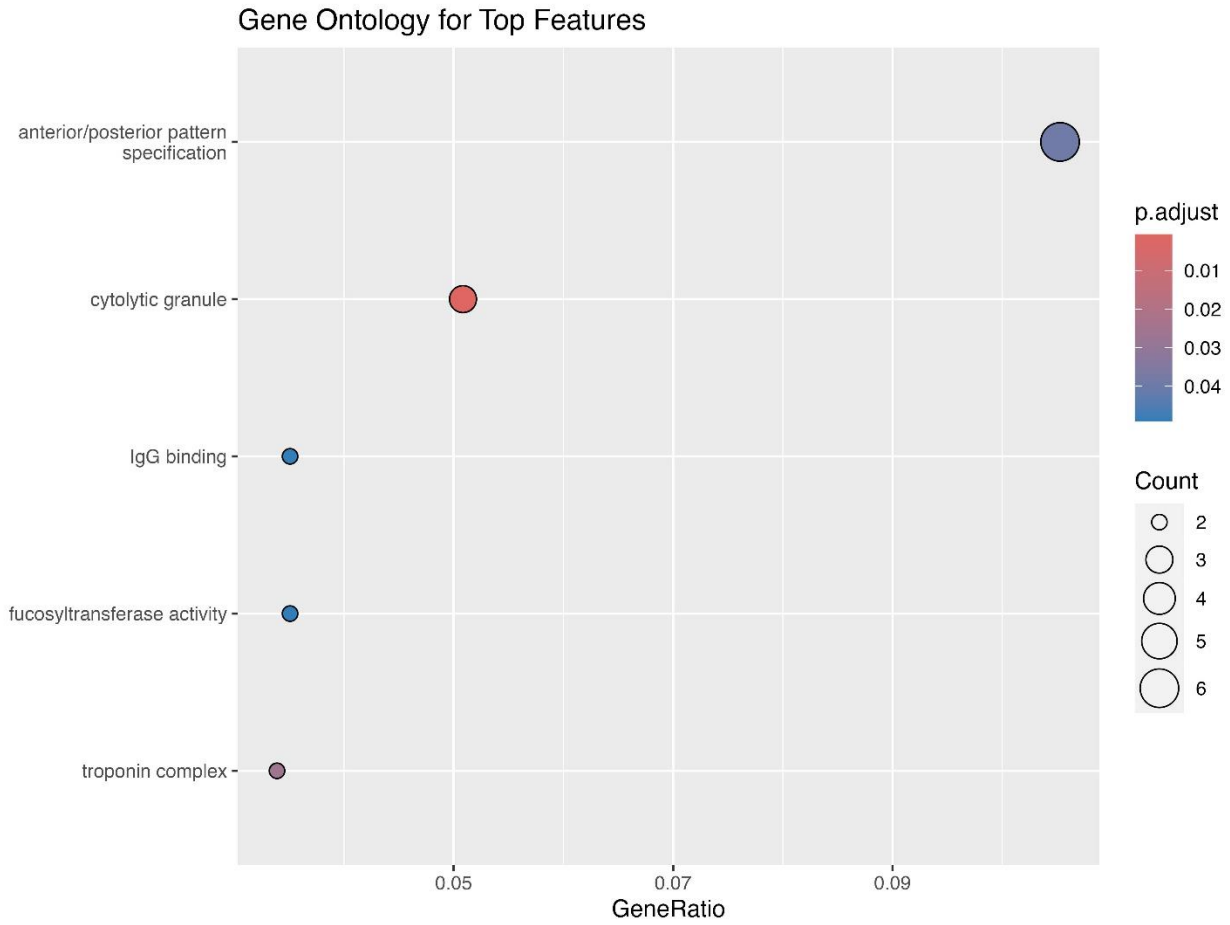
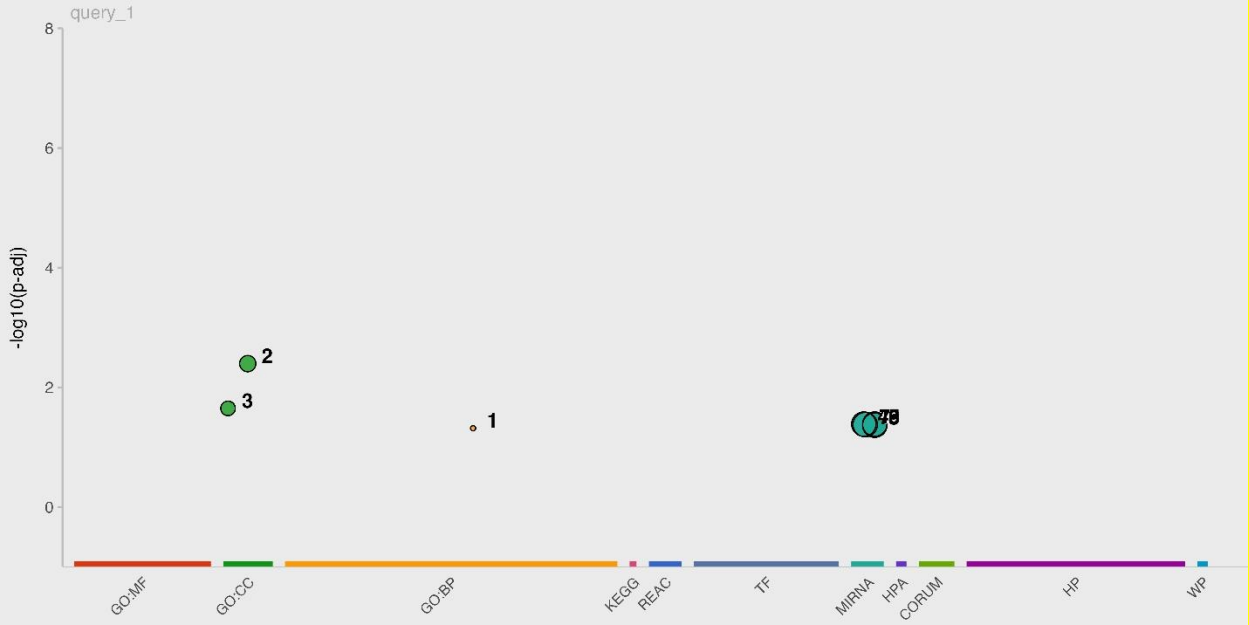


Figure 3. Gene Ontology enrichment analysis using *clusterProfiler* on the significant features identified by *pyRforest*. The x-axis represents the GeneRatio, the proportion of genes involved in the GO term relative to the total number of genes studied. The y-axis lists the GO terms associated with the identified features. Circle size indicates the gene count associated with each term, while the color gradient represents the adjusted p-value (p.adjust), with cooler colors indicating higher significance levels.



id	source	term_id	term_name	term_size		p_value
1	GO:BP	GO:0060743	epithelial cell maturation involved in prostate gland development	3		4.8e-02
2	GO:CC	GO:0044194	cytolytic granule	14		4.0e-03
3	GO:CC	GO:0005861	troponin complex	9		2.2e-02
4	MIRNA	MIRNA:hsa-miR-4419a	hsa-miR-4419a	298		4.1e-02
5	MIRNA	MIRNA:hsa-miR-6130	hsa-miR-6130	298		4.1e-02
6	MIRNA	MIRNA:hsa-miR-6129	hsa-miR-6129	299		4.2e-02
7	MIRNA	MIRNA:hsa-miR-4510	hsa-miR-4510	300		4.2e-02
8	MIRNA	MIRNA:hsa-miR-6127	hsa-miR-6127	302		4.3e-02
9	MIRNA	MIRNA:hsa-miR-6133	hsa-miR-6133	302		4.3e-02

g:Profiler (biit.cs.ut.ee/gprofiler)

Figure 4. Enrichment analysis Manhattan plot from *g:Profiler* calculated on the 83 significant features found in our CRC case study. The x-axis categorizes enriched terms from various databases, and the y-axis shows their significance (-log10 p-value). Points represent terms, with notable significantly enriched terms named in the table.

clusterProfiler and *g:Profiler* Analysis

pyRforest returns a list of genes that can easily be used as input for the R packages *clusterProfiler* and *g:Profiler*. The *clusterProfiler* gene ontology (GO) enrichment analysis of the 83 identified features, revealed involvement in significant biological processes such as ‘anterior/posterior pattern specification’ and ‘cytolytic granule’ **(Figure 3)**. The top results from *g:Profiler* gene enrichment analysis also reinforce the findings that there is a potential link between prostate development processes and CRC anatomical side **(Figure 4)**. The enriched *GO* terms provide biological plausibility to the findings of the case study, indicating that the significant features identified by *pyRforest* are not just statistically relevant but also biologically grounded in the context of CRC lateralization.

Discussion

pyRforest combines the computational power of Python and the statistical and genomic analysis capabilities of R into one package, addressing a crucial need in modern genomic research. Moreover, *pyRforest* offers a range of extra utility functions for biomarker selection and analysis. These include (1) a rank-based permutation approach which allows the user to identify the features that have statistically significant importance scores, (2) integration with the Python module `SHAP.TreeExplainer` for feature interpretation and (3) compatibility with *clusterProfiler* and *g:Profiler* for producing gene enrichment results which allow the user to explore the list of significantly important features in the context of external databases of known biological functions.

One primary objective of analyzing genomic data with RF models is to discern important biomarkers. We present a comparison of *pyRforest* with two other packages which each differ in their approach to using permutation to identify important features. The `permutation_importance` function in the *scikit-learn* `sklearn.inspection` module [7] independently permutes features, which is computationally intensive and destroys any associations among features. In contrast, *pyRforest* permutes only the response variable, thereby leaving the relationships among features intact. This approach offers advantages in computational speed, and we consider this approach to yield a more appropriate null model, given the extent of correlations present within real genomic data and known gene-gene interactions. Another library, *rfPermute* [19], is a wrapper for the *randomForest* package. Like *pyRforest*, *rfPermute* permutes only the target variable and not the features; however, it compares the importance of each feature to its own distribution of importances scores under a permuted target variable. In contrast, *pyRforest* compares each observed feature importance to the distribution of permuted importance scores at its rank, rather than to the distribution of permuted importance scores of the feature itself. The null distribution for the most important feature (rank 1) is the scores obtained at rank 1 under permutation, regardless of the possibility of the identity of the specific feature changing with each permutation.

We consider that these two properties, permuting the target variable and not the features, and using rank-based importance distributions offers a more appropriate null model and basis for comparing observed importance scores, and, ultimately, identifying a reduced set of potentially important features.

The approach of *pyRforest* also leads to two key effects that differentiate it from other rank-based methods, such as that of the previously mentioned *rfPermute* [19], and the approach implemented in Altmann et al. [20], which determines feature significance by comparing the rank of a feature in the test data to the distribution of ranks in permuted data. First, *pyRforest* can select important variables for datasets in which a large proportion of variables play a role. For example, in a dataset with 100 variables, all things being equal, a variable will have an importance rank of 50 in a permuted dataset (with a standard deviation of approximately 7 under

a Poisson distribution). However, if 50 or more variables out of 100 are predictive, the 50th variable will not have a rank importance greater than its average in the permuted data. However, in *pyRforest*, the importance of the 50th variable may be considerably higher than the average in a permuted dataset. Second, *pyRforest* is more attuned to feature importances taking extreme values, especially in permuted datasets. Such values may be uncommon, but key for deciding whether a feature is important for an RF model. For feature-rank based approaches, large outlier values are obscured, as the approach is inherently non-parametric in deciding the deviation of feature importance compared to a null distribution.

In addition, the *pyRforest* rank-based approach forgoes the need to permute and evaluate each feature individually, and this generally offers improved speed compared to feature-based approaches.

Despite its innovative contributions, the *pyRforest* R package comes with limitations worth noting. The primary limitation is computation time, although *pyRforest* is considerably faster than other packages. However, this could be improved by fitting a theoretical probability distribution to an empirical distribution derived from only a small number of permutations [20]. Continued and improved integration of advanced analytical features, such as the ability to handle multi-class classification problems, would enable *pyRforest* to further expand its utility and accessibility.

To demonstrate the utility of *pyRforest* as an analytical tool, we have presented a case study of a dataset previously analyzed in Kolisnik et al. (BMC Cancer, 2023) [22]. We identified 83 significant biomarkers, in contrast with 15 reported in the previous study [22]. This provides a more optimal number of biomarkers for statistical significance calculations in gene-set enrichment studies. In addition to this, integration with SHAP values provides new insights into the effects of each feature on outcomes. Our rank-based permutation approach was also applied in a study by Keshavarz-Ragahi et al. *(includes Kolisnik)* [27] to identify multiple key features associated with p53 activity, enhancing the understanding of TP53-related transcriptional signatures across various cancer types.

In summary, *pyRforest* offers a range of tools to fit, interpret and contextualize the results of RF models for genomic studies by combining Python and R. *pyRforest* represents a step forward in streamlining and enriching genomic data analysis. We encourage the research community to utilize, contribute to, and build upon *pyRforest* for advancing ML-based genomic analyses.

Key Points

- We developed *pyRforest*, an R package which offers seamless integration of *scikit-learn* `RandomForestClassifier`, `GridSearchCV`, and `shap.TreeExplainer` modules into the R ecosystem via *reticulate*.

- *pyRforest* introduces a rank-based feature importance permutation test designed for biomarker identification, which prioritizes and assigns statistical importance to RF model features based on Gini Importance scores.
- *pyRforest* facilitates the exploration of the underlying biological mechanisms of RF models through *ggplot2* visualizations, and downstream integration with *clusterProfiler* and *g:Profiler*.
- We demonstrate the effectiveness and utility of *pyRforest* in a colorectal cancer case study, where it identifies key biomarkers previously known to the literature and provides insights into the underlying biological mechanisms of the RF model.
- Download, collaborate, make suggestions, or report bugs at: https://www.github.com/tkolisnik/pyRforest.

Supplementary Material

Supplementary data is available online at www.biorxiv.com

Author Statements

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Authors' contributions

TK conceived the project, wrote the code and documentation for the R package, performed testing, analysis of results and figure generation, and wrote the first draft and contributed to all versions of the manuscript. AS guided the development of statistical methods, providing validation and proof of concept, manuscript proof-reading, as well as guided presentation of results. OKS provided support with development, troubleshooting, statistics, and critical analysis of results, and contributed to the manuscript and write-ups. FK assisted with testing of the *pyRforest* package and manuscript proof-reading. All authors have read and approved this manuscript.

Data Availability

Raw Sequence Reads are available at SRA BioProject ID: PRJNA788974 [28].

Code Availability

The R package *pyRforest* used in these analyses is available at: www.github.com/tkolisnik/pyRforest [16].

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Ethics Approval and consent to participate

This study was approved by the University of Otago, New Zealand, Human Research Ethics Committee (approval number: H16/037). Informed consent was obtained from all subjects and/or their legal guardians. All experiments were performed in accordance with relevant ethics guidelines and the Declaration of Helsinki.

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