

# iMIRAGE Vignette

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## Introduction

**iMIRAGE** stands for *imputed microRNA (miRNA) activity from gene expression*. As the name suggests, the package imputes expression of miRNAs by constructing prediction models that only depend on the expression levels of protein-coding genes. In essence, iMIRAGE package can impute the miRNA profiles of samples where protein-coding expression data is available (for example, from microarray or RNA-seq), but do not contain reliable miRNA expression. By utilizing a *training* data set containing both protein-coding and miRNA expression profiles, iMIRAGE trains and predicts miRNA expression in the *independent (test)* data set of interest.

The iMIRAGE package also provides tools to create an integrated workflow, to harmonize, clean-up, normalize and standardize the expression data sets. In addition, the package provides an option of using miRNA-target gene pair information to construct the prediction models

## Download and installation

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The iMIRAGE package for R can be downloaded from the [GitHub repository](#):

```
#Download package from GitHub using devtools
devtools::install_github("aritrnath/iMIRAGE")
```

```
#Load the package in R
library(iMIRAGE)
#> Loading required package: randomForest
#> randomForest 4.6-14
#> Type rfNews() to see new features/changes/bug fixes.
#> Loading required package: FNN
#> Loading required package: e1071
```

## Whats included in this package?

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The iMIRAGE package includes necessary functions for pre-processing and harmonization of expression data sets, miRNA expression imputation and cross-validation analyses. In addition, TargetScan miRNA-target gene pairs are provided for constructing imputation models. The package also includes the example data sets that are used in this vignette.

## Report issues

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[Report bugs and issues here via GitHub](#)

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## Quick Start Guide

In this example, we will use the two **independent** miRNA data sets that were derived from the Cancer Genome Atlas (TCGA) Breast Cancer (BRCA) project. These data sets are automatically available when the iMIRAGE

package library is loaded.

## Datasets:

1. Training: *GA.pcg*, *GA.mir*
  - The prefix *GA* refers to TCGA breast invasive carcinoma (BRCA) samples for which miRNA mature strand expression were obtained using the Illumina Genome Analyzer system.
2. Test: *HS.pcg*, *HS.mir*
  - The prefix *HS* refers to TCGA BRCA samples for which miRNA mature strand expression were obtained using the Illumina Hiseq system.
  - The samples in the two data sets are mutually exclusive. The small subset included in these example data sets were randomly selected
  - Note: the complete TCGA BRCA HiSeq and GA data sets can be downloaded from the [XENA portal](#)

## Step 1: Match protein-coding genes (predictive features) in the training and test datasets with `match.gex`

```
# return a pair of matrices with matched columns (protein-coding genes that will be used as training
features)

temp <- match.gex(GA.pcg, HS.pcg)
GA.pcg <- temp[[1]]
HS.pcg <- temp[[2]]
```

## Step 2A: Perform cross-validation analysis with `imirage.cv` to determine the expected accuracy of imputing a miRNA of interest using the training datasets

```
#Use **imirage.cv** with the default parameters of using K-nearest neighbors as the prediction
algorithm and using 50 protein-coding genes as training features

CV.miRNA <- imirage.cv(train_pcg=GA.pcg, train_mir=GA.mir, gene_index="hsa-let-7c", method="KNN",
target="none")
#>
#> Running 10-folds cross-validation...
#> Iteration 1
#> Iteration 2
#> Iteration 3
#> Iteration 4
#> Iteration 5
#> Iteration 6
#> Iteration 7
#> Iteration 8
#> Iteration 9
#> Iteration 10
#> Cross-validation complete

#Print the accuracy metrics from each fold of the cross-validation analysis
print(CV.miRNA)
#>      PCC      P-Value      RMSE
#> [1,] 0.8779923 0.000000e+00 1782.076
#> [2,] 0.4789272 1.068270e-02 1542.090
#> [3,] 0.6588235 2.676593e-05 2184.594
#> [4,] 0.8990148 4.541760e-07 1903.875
#> [5,] 0.7567568 4.645242e-07 1243.088
#> [6,] 0.5740026 3.222117e-04 2386.052
```

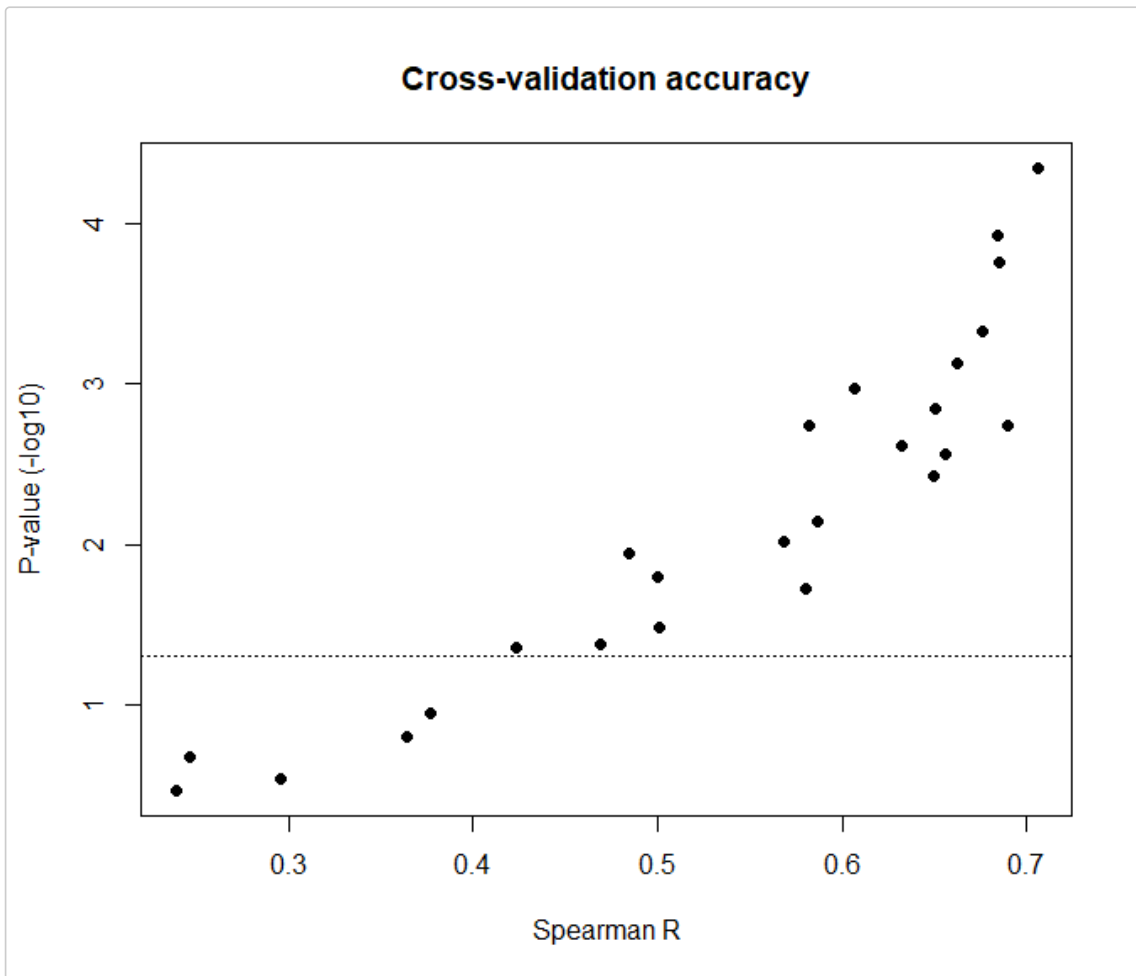
```
#> [7,] 0.5889328 3.692481e-03 1897.031
#> [8,] 0.7541812 1.444771e-07 1717.641
#> [9,] 0.6985843 4.426766e-06 1047.888
#> [10,] 0.7018910 2.574267e-05 1421.781
```

*#note: this example performs cross-validation analysis for 1 unique miRNA, hsa-Let-7c. The name must match with one of the names in the train\_mir object's column names*

## Step 2B: Perform cross-validation analysis with `imirage.cv.loop` to determine the expected imputation accuracy of entire miRNA dataset with

```
#Perform cross-validation analysis over the entire training dataset
CV.full <- imirage.cv.loop(train_pcg = GA.pcg, train_mir = GA.mir, method = "KNN", target="none")

#Plot some performance metrics from cross-validation analysis
plot(CV.full[,1], -log10(CV.full[,2]), xlab="Spearman R", ylab="P-value (-log10)", pch=16, main="Cross-
validation accuracy")
abline(h=-log10(0.05), lty=3)
```



```
#Find out which miRNAs are imputed with good accuracy
colnames(GA.mir)[which(CV.full[,1] > 0.5)] #arbitrarily, Spearman correlation > 0.5
#> [1] "hsa-Let-7c" "hsa-mir-100" "hsa-mir-10b" "hsa-mir-1301"
#> [5] "hsa-mir-1307" "hsa-mir-130b" "hsa-mir-141" "hsa-mir-150"
#> [9] "hsa-mir-155" "hsa-mir-17" "hsa-mir-18a" "hsa-mir-210"
#> [13] "hsa-mir-301a" "hsa-mir-3677" "hsa-mir-4772" "hsa-mir-99a"
```

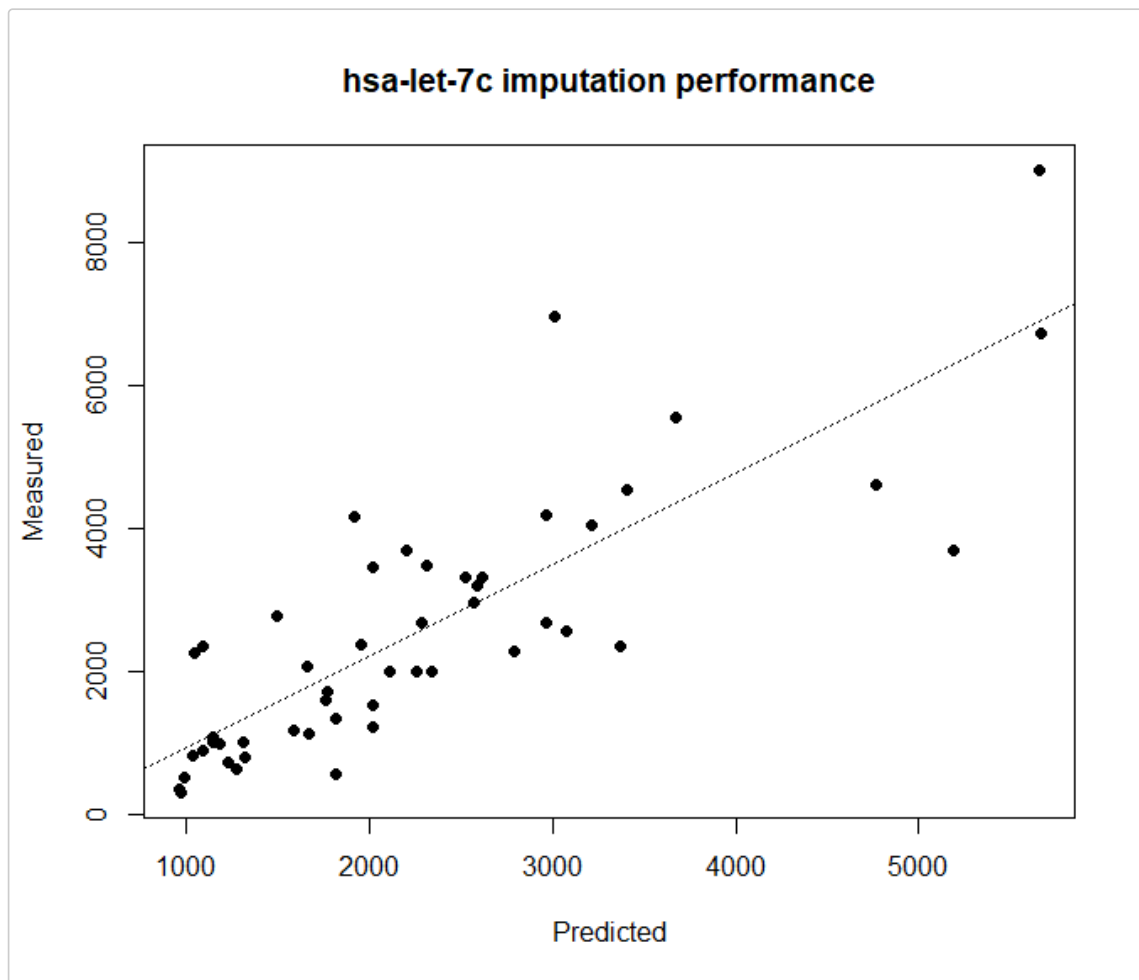
```
colnames(GA.mir)[which(CV.full[,2] < 0.05)] #P-value < 0.05
#> [1] "hsa-let-7c" "hsa-mir-100" "hsa-mir-10a" "hsa-mir-10b"
#> [5] "hsa-mir-1301" "hsa-mir-1307" "hsa-mir-130b" "hsa-mir-141"
#> [9] "hsa-mir-146a" "hsa-mir-150" "hsa-mir-155" "hsa-mir-17"
#> [13] "hsa-mir-18a" "hsa-mir-190b" "hsa-mir-20a" "hsa-mir-210"
#> [17] "hsa-mir-301a" "hsa-mir-3677" "hsa-mir-4772" "hsa-mir-99a"
```

## Step 3A: Impute expression of a single miRNA of interest using `imirage` in the test dataset

```
#Use **imirage** with default parameters
Pred.miRNA <- imirage(train_pcg=GA.pcg, train_mir=GA.mir, my_pcg=HS.pcg, gene_index="hsa-let-7c",
target="none")

#Display the predicted miRNA expression values
print(Pred.miRNA)
#> [1] 2019.3563 1498.5680 1771.0472 2525.3074 3406.9625 1813.6320 3009.6051
#> [8] 2198.6933 3072.8239 1307.7639 2962.8586 1277.5310 2258.6073 2110.7417
#> [15] 5194.3184 2963.7463 1765.5263 3669.1882 1952.3878 3368.4370 1316.7190
#> [22] 2017.3545 1671.8867 5657.6180 1037.8135 3214.5502 5665.0500 1815.9127
#> [29] 2612.9871 2567.6086 2341.5214 2787.6283 2312.1913 1230.4309 2020.4602
#> [36] 969.7257 1145.1208 958.9258 1043.2435 1181.7525 1092.2694 2585.8381
#> [43] 4762.9124 1914.3931 1659.7709 2286.6204 1590.2248 987.3592 1146.6056
#> [50] 1086.8198

#Compare the predicted miRNA expression values with measured expression
plot(Pred.miRNA, HS.mir[, "hsa-let-7c"], xlab="Predicted", ylab="Measured", main="hsa-let-7c imputation
performance", pch=16)
abline(lm(HS.mir[, "hsa-let-7c"] ~ Pred.miRNA), lty=3)
```



### Step 3B: Use `imirage` to impute expression of all miRNAs available in the training dataset

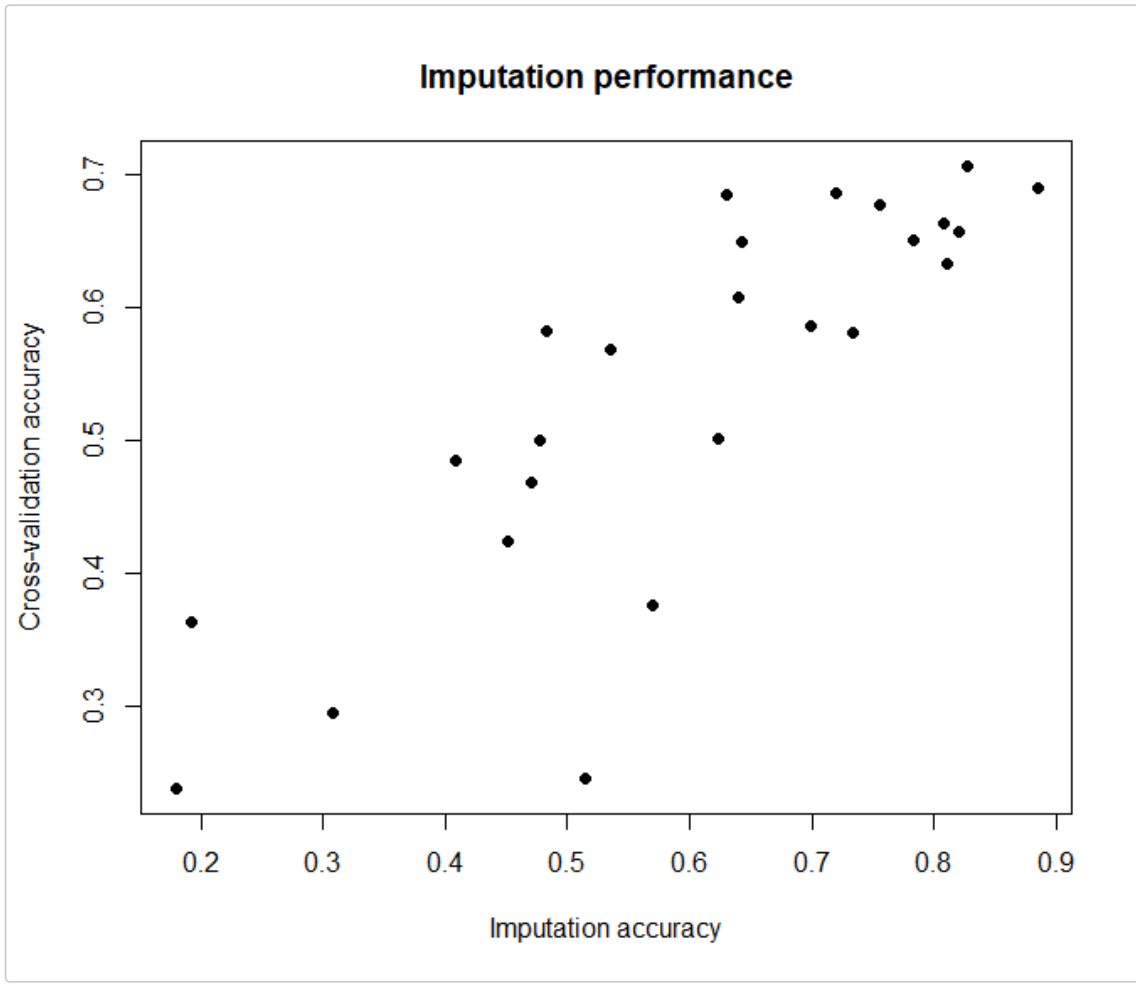
```
#Create an empty matrix to store imputed expression
Pred.full <- matrix(data=NA, ncol=ncol(GA.mir), nrow=nrow(HS.pcg))

#Execute a loop to impute each miRNA using the test protein coding dataset
for (i in 1:ncol(GA.mir)) {
  Pred.full[,i] <- imirage(train_pcg = GA.pcg, train_mir = GA.mir, my_pcg = HS.pcg, gene_index = i,
    method="KNN", target="none")
}
```

Find out how well can we impute miRNA expression in the **independent** data set

```
#Obtain correlation coefficients between imputed and measured miRNA expression
Pred.Cors <- array(dim=ncol(GA.mir))
for (i in 1:ncol(GA.mir)) {
  Pred.Cors[i] <- cor(HS.mir[,i], Pred.full[,i], method="spearman")
}

#Plot imputation correlations in comparison to cross-validation results
plot(Pred.Cors, CV.full[,1], xlab="Imputation accuracy", ylab="Cross-validation accuracy",
  main="Imputation performance", pch=16)
```



## Detailed guide

### Background

The principle behind iMIRAGE can be explained in a simplified form by stating the problem in the form of a linear model. Let us assume the user wants to obtain miRNA expression using their protein-coding data set which we will all *test*. To impute the miRNA expression using the *test* protein-coding data set, we first determine the mathematical relation between the expression of a set of miRNAs  $Y$  with the set of protein-coding gene expression  $X$  of the *training* data set, where  $p$  indicates the number of protein-coding genes used in the model. This number can range anywhere from 1 to  $P$ , where,  $P = \text{total number of protein-coding genes available in both the training and test data sets}$ .

$$Y^{Training} = \sum_{i=1}^p \beta X_i^{Training} + \epsilon$$

The objective of iMIRAGE is to determine  $\beta$  so that it can be harnessed to predict the expression of miRNA  $Y$  by applying the model to the *test* protein-coding data set:

$$Y^{Predicted} = \sum_{i=1}^p \beta X_i^{Test}$$

(Note that the machine-learning algorithms that are used iMIRAGE are not necessarily based on the assumption of linearity. This is only for simplified illustration purposes)

iMIRAGE assumes that not all protein-coding genes are meaningful for constructing imputation models. Therefore, it suggests using a subset of informative protein-coding genes for each miRNA. These informative features are automatically determined by calculating the correlation between the expression of all features with miRNA expression in the training data sets. Then, only a user-specified subset of the top correlated features are retained for constructing the models (by default 50).

## Required data

1. Training data: protein-coding and miRNA expression data. In the package documentation, these data sets are referred by their alias *train\_pcg* and *train\_mir* respectively
  - Both data sets must be from the same samples
  - High-quality miRNA expression data - from small RNA-seq or miRNA arrays preferred
  - miRNA expression from regular RNA-seq libraries are not reliable
2. Protein-coding gene expression data from the samples of interest. In the package documentation, this data set is referred by its alias *my\_pcg*
3. Organizing your data before use:
  - Your data should be arranged as a  $n \times p$  matrix, where  $n$  denotes samples in *rows* and  $p$  denotes genes/miRNA in *columns*
  - Row names should be names of the samples
  - Column names should be names of the gene/miRNA
  - Gene IDs should be of the same type in the training and test data sets
  - If you plan to use target gene pairs for constructing imputation models, the miRNA nomenclature should match between the training miRNA data set and the miRNA-target gene pairs. Similarly, the protein-coding gene IDs in the training and test data sets should match with the type of ID used by the miRNA-target gene pair data set
  - To convert gene IDs, use the R/Bioconductor package [biomaRt](#)

```
#Short example on converting RefSeq mRNA IDs to ENSEMBL IDs using biomaRt
library(biomaRt)
ensembl = useMart("ensembl",dataset="hsapiens_gene_ensembl")
old.ids <- colnames(my_pcg) #RefSeq IDs
gene.annot <- getBM(attributes=c('refseq_mrna','ensembl_gene_id'),
                    filters = 'refseq_mrna',
                    values = as.character(old.ids),
                    mart = ensembl)

#returns a dataframe with ENSEMBL IDs corresponding to supplied RefSeq mRNA IDs
```

[See the biomaRt documentation for complete details and instructions](#)

## Workflow

We will use the unprocessed TCGA BRCA data sets to illustrate a typical workflow. The data set used in the subsequent examples can be downloaded from the Open Science Framework repository by following the following URL: <https://osf.io/s5rbn/download>

After downloading the TCGA\_BRCA\_Datasets.RData file in the current working directory, load the contents using

```
load("TCGA_BRCA_Datasets.RData")
```

The following four matrices should appear after the object is loaded: ga.gex, ga.mirna, hiseq.gex, hiseq.mirna

### 1. Filtering training datasets using `filter.exp` to remove gene or miRNAs that are not expressed in most samples using (optional)

We first remove gene or miRNAs that are not expressed in most samples, as these genes are likely not measured due to technical limitations and may not be reliable expression estimates. Ultimately, it is up to the user to decide whether they would like to include the sparse genes or miRNA in their analyses.

```
#Here, we filter the miRNA datasets to retain all miRNAs that are expressed above a level of 0 in
atleast 75% of the samples
ga.mirna <- filter.exp(ga.mirna, cutoff=75, threshold = 0)
hiseq.mirna <- filter.exp(ga.mirna, cutoff=75, threshold = 0)
```

## 2. Use `match.gex` to match training and test protein-coding expression matrices (required)

Here, we match the two protein-coding expression matrices for subsequent processing

```
#We also will keep the unprocessed hiseq.gex.1 and ga.gex.1 datasets for subsequent comparisons
temp <- match.gex(hiseq.gex, ga.gex)
hiseq.gex.1 <- temp[[1]]
ga.gex.1 <- temp[[2]]

#For subsequent prediction analyses, we will also select miRNAs are common to both datasets
temp <- match.gex(ga.mirna, hiseq.mirna)
ga.mirna.1 <- temp[[1]]
hiseq.mirna.1 <- temp[[2]]
```

## 3. pre.process training and test datasets (optional, recommended)

In this step, we process the training and test data sets using `pre.process` to make sure they are transformed, normalized and standardized before proceeding with further analysis. These steps tend to have a significant impact on subsequent analyses.

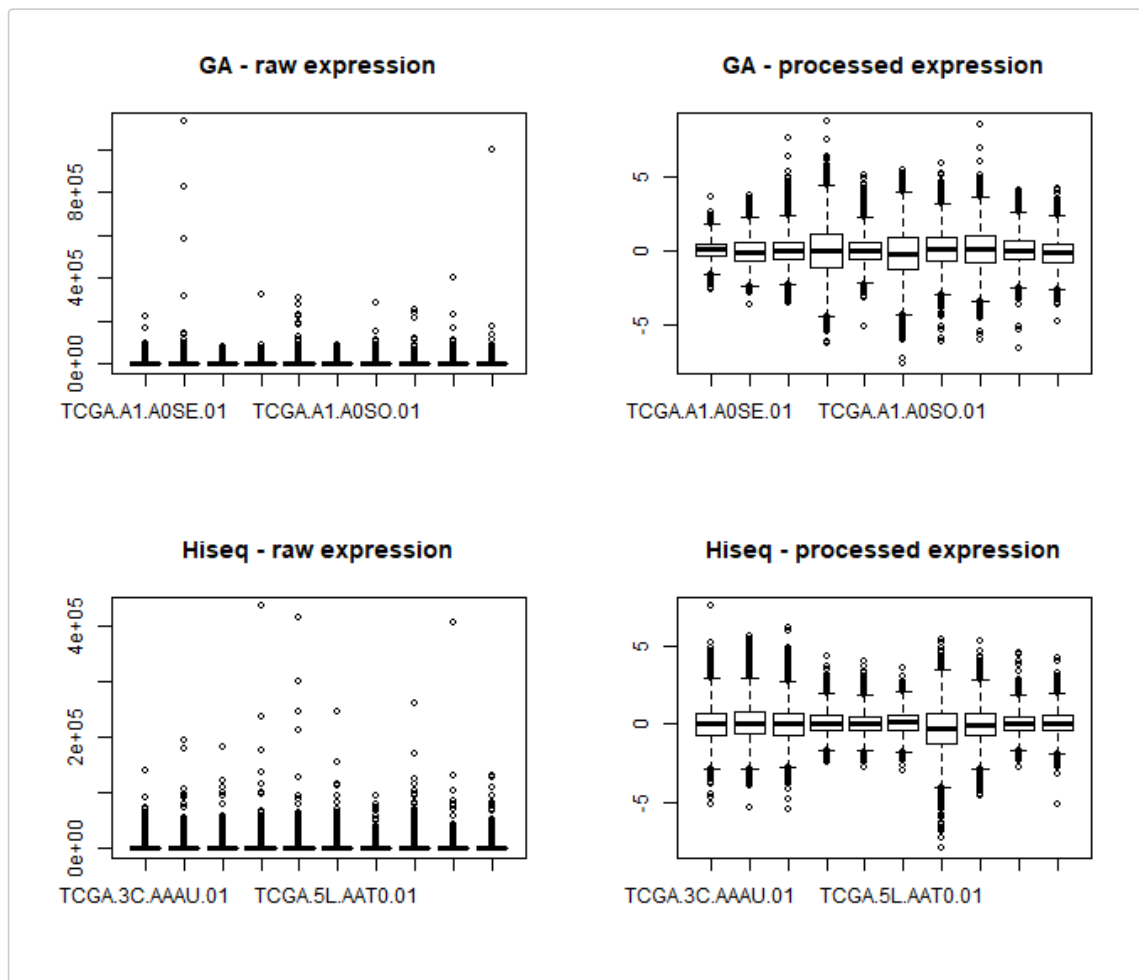
Generally, the user should determine whether their data has been transformed ( $\log 2$  or  $\log 2(x + 1)$ ) or normalized from the original source. In most cases, microarray data sets in public domain, such as [NCBI GEO](#) or [ArrayExpress](#), are already transformed and normalized. However, several RNA-seq data sets may be available prior to transformation and normalization. In these instances, it is advisable to perform log transformation and upper-quantile normalization prior to use in the workflow.

In addition, the variance filter removes genes with zero variance that will offer no predictive power. Finally, the data is scaled to a mean = 0 and standard deviation = 1. These are bare-minimum standardization techniques which can be skipped if the user has already pre-processed their data using other approaches.

```
ga.gex.2 <- pre.process(ga.gex.1, log = TRUE, var.filter = TRUE, UQ = TRUE, std = TRUE)
hiseq.gex.2 <- pre.process(hiseq.gex.1, log = TRUE, var.filter = TRUE, UQ = TRUE, std = TRUE)

#This is how the data looks before and after pre.processing (showing the first 10 samples from each dataset)
par(mfrow=c(2,2), cex=0.75)
boxplot(t(ga.gex.1[1:10,]), main="GA - raw expression")
boxplot(t(ga.gex.2[1:10,]), main="GA - processed expression")
boxplot(t(hiseq.gex.1[1:10,]), main="Hiseq - raw expression")
boxplot(t(hiseq.gex.2[1:10,]), main="Hiseq - processed expression")
```





#### 4. Performing cross-validation to obtain accuracy metrics and imputing in test dataset

Here, we will first find out which miRNAs can be imputed with good accuracy using the GA data set as our training data set. The `imirage.cv` function performs a 10-fold cross-validation analysis for a single miRNA specified by the `gene_index` argument, as shown in the quick start guide above. In addition, the `imirage.cv.loop` wrapper can be used to perform the cross-validation analysis on the entire training miRNA data set.

The cross-validation analysis can be performed using one of the three methods: K-nearest neighbor regression (KNN), Random Forests (RF) or Support Vector Machines (SVM). These can be specified using the `method` argument.

For further details on the arguments that can be passed on the machine-learning methods, please see the documentation for [randomForest](#), [e0171](#) and [FNN](#) packages.

By default, the number of training features that are used by each method are set at 50. This can be adjusted by the user using the `num` argument to achieve a balance between desired imputation accuracy and computational costs.

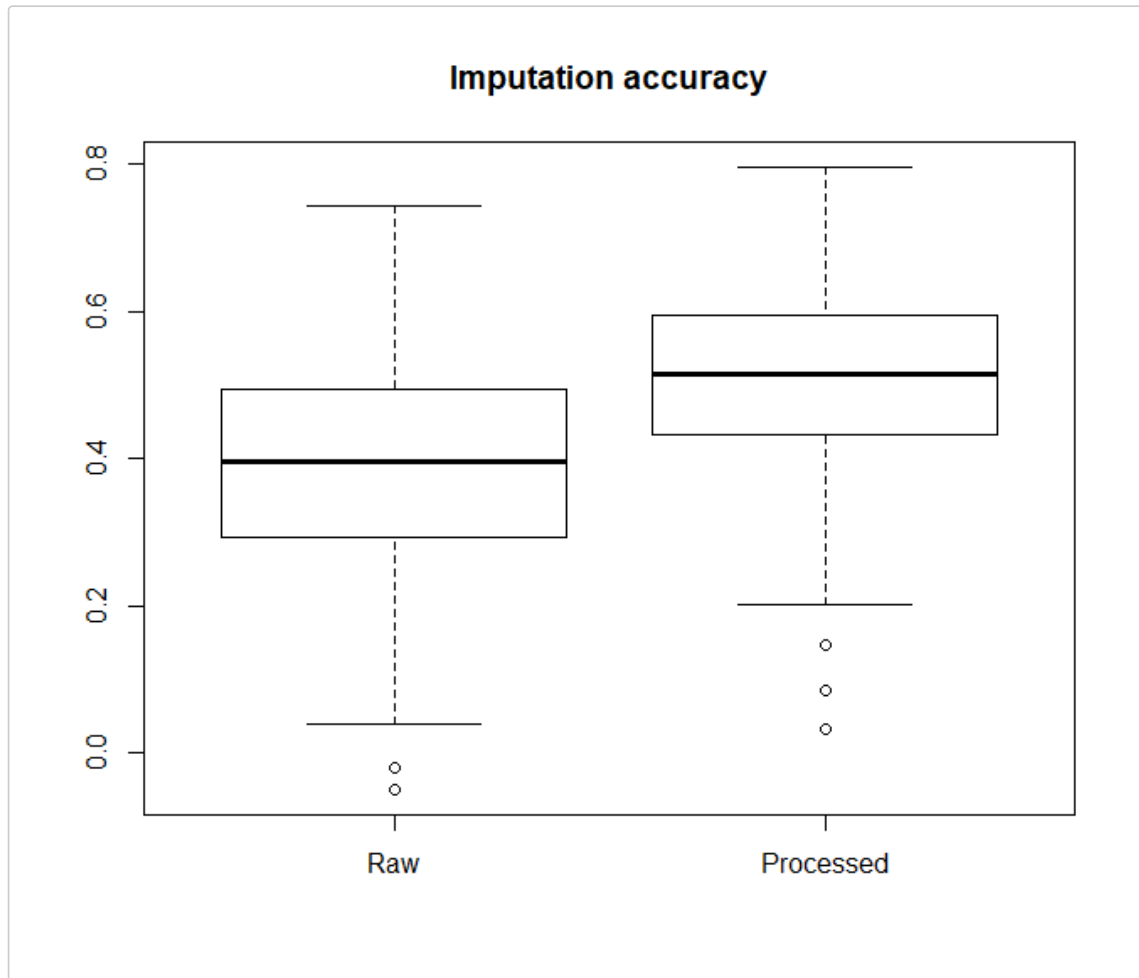
Additionally, the “K” or number of cross-validation iterations can be set using `folds`, which is set at 10 by default.

Here, we perform a 10-fold cross-validation analysis using the entire GA training data sets using KNN method. We perform the analysis with both the processed and unprocessed GA protein-coding data for comparison

```
#Unprocessed training data
CV.ga.gex1 <- imirage.cv.loop(train_pcg = ga.gex.1, train_mir = ga.mirna.1, method = "KNN",
target="none")

#Processed training data
CV.ga.gex2 <- imirage.cv.loop(train_pcg = ga.gex.2, train_mir = ga.mirna.1, method = "KNN",
target="none")

#Comparison of imputation accuracies between raw and processed data
boxplot(CV.ga.gex1[,1], CV.ga.gex2[,1], names=c("Raw", "Processed"), main="Imputation accuracy")
```



Subsequently, the user can either select the miRNAs with good performance metrics in cross-validation analysis or perform the imputation using the full training data set with `imirage`. Generally, poor cross-validation metrics are a good indicator that the miRNA can be safely excluded from the prediction analysis in the independent test data set.

## Session Info

```
sessionInfo()
#> R version 3.6.1 (2019-07-05)
#> Platform: x86_64-w64-mingw32/x64 (64-bit)
#> Running under: Windows 10 x64 (build 17763)
#>
#> Matrix products: default
#>
#> Locale:
#> [1] LC_COLLATE=English_United States.1252
#> [2] LC_CTYPE=English_United States.1252
#> [3] LC_MONETARY=English_United States.1252
#> [4] LC_NUMERIC=C
#> [5] LC_TIME=English_United States.1252
#>
#> attached base packages:
#> [1] stats    graphics  grDevices utils      datasets  methods   base
#>
#> other attached packages:
#> [1] iMIRAGE_1.1      e1071_1.7-2      FNN_1.1.3
```

```
#> [4] randomForest_4.6-14
#>
#> loaded via a namespace (and not attached):
#> [1] Rcpp_1.0.1      compiler_3.6.1  prettyunits_1.0.2
#> [4] remotes_2.1.0   class_7.3-15    tools_3.6.1
#> [7] testthat_2.1.1  digest_0.6.20   pkgbuild_1.0.3
#> [10] pkgload_1.0.2   memoise_1.1.0   evaluate_0.14
#> [13] rlang_0.4.0     cli_1.1.0       rstudioapi_0.10
#> [16] yaml_2.2.0      xfun_0.8        withr_2.1.2
#> [19] stringr_1.4.0   knitr_1.23      desc_1.2.0
#> [22] fs_1.3.1        devtools_2.1.0  rprojroot_1.3-2
#> [25] glue_1.3.1      R6_2.4.0        processx_3.4.0
#> [28] rmarkdown_1.14  sessioninfo_1.1.1 callr_3.3.0
#> [31] magrittr_1.5    backports_1.1.4 ps_1.3.0
#> [34] htmltools_0.3.6 usethis_1.5.1   assertthat_0.2.1
#> [37] stringi_1.4.3   crayon_1.3.4
```