Ancestry estimation based on reference samples of known ethnicities

Maha Syed and Caroline Walter

2025-11-16

Contents

Running Random Forest Classification

This package contains a function to predict ancestry of samples. This works through a random forest algorithm trained on the principal components of cleaned genomics data from the 1000 genomes dataset. This method is based off the ancestry identification scheme used in the Pan-UK BioBank analysis.

Plink v2 is needed for this portion of the package. Additional loading matrices are needed for the PCA projection used in the model. This is hosted on the plinkQC github repo under the inst/extdata folder located here. Alternatively, the whole github repo can downloaded with

```
git clone git@github.com:meyer-lab-cshl/plinkQC.git
```

Workflow

To begin with this is an example of how to run the classifier with a provided test data set. We will set up a temporary directory for qcdir.

```
package.dir <- find.package('plinkQC')
indir <- file.path(package.dir, 'extdata')
qcdir <- tempdir()
name <- 'data.hg38'
path2plink2 <- "/Users/syed/bin/plink2"
path2load_mat <- "/Users/syed/Documents/loading_matrix"
```

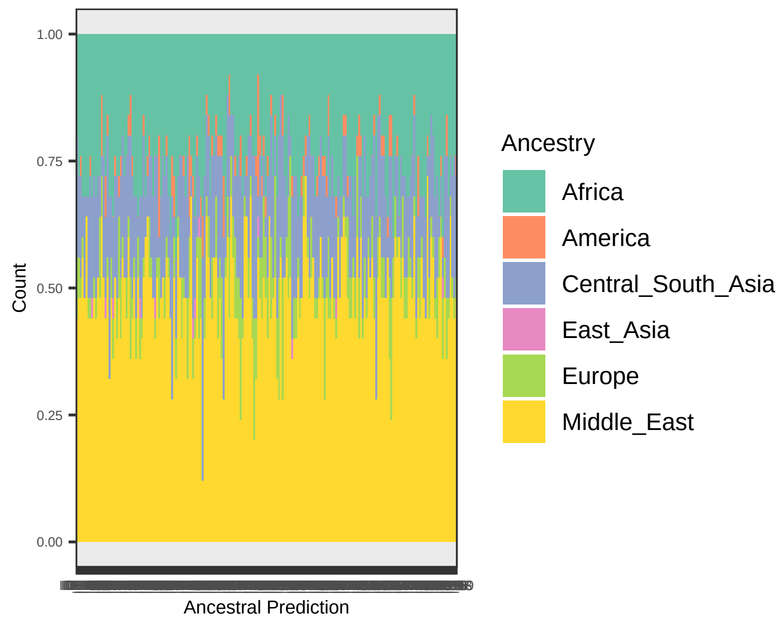
Before use, the study data should be in the new hg38 annotation. USCS's liftOver tool may be needed to map variants from one annotation to another. More details on how to use the tool can be found on the processing HapMap III reference data vignette.

In addition, the data should be in PLINK 2.0 format (i.e pgen, pvar, psam files), and the variants identifiers should be formatted similar to the following example: 1:12345[hg38]. We can use the included functions convert_to_plink2() and rename_variant_identifiers() to correctly format genomic data that is in the hg38 annotation.

```
convert_to_plink2(indir=indir, qcdir=qcdir, name=name,
                  path2plink2 = path2plink2)
rename_variant_identifiers(indir=qcdir, qcdir=qcdir, name=name,
                           path2plink2 = path2plink2)
name <- paste0(name, ".renamed")
```

After correctly formatting the variants, we can use the provided function to return the predicted ancestry. Because the example dataset has only a small number of variants overlapping with the reference dataset, additional noise is expected.

```
ancestries <- superpop_classification(indir=qcdir, qcdir=qcdir, name=name,
                                      path2plink2 = path2plink2,
                                      path2load_mat = path2load_mat)
ancestries$p_ancestry
```



Training Your Own Random Forest Classifier

Based on the microchip used in the study data, there might not be a large enough overlap in the SNPs sequenced to gain accurate predictions. In this case, we recommend training your own random forest. To do so, we will first need to set up the needed bash variables and download the 1000 genomes dataset into qcdir which is described in detail in Processing 1000 Genomes reference data for ancestry estimation. We will use a test study dataset included in the package as an example for the workflow.

Setting up Bash Script

```
qcdir='~/qcdir'
name='data.hg38'
refdir=~/reference

mkdir -r $qcdir/plink_log
```

Putting study data in qcdir

Copy your study dataset to the qcdir folder. In this example, we are assuming that the provided test dataset included with the package has been moved.

Workflow

Download reference data

A suitable reference dataset should be downloaded and if necessary, re-formated into PLINK format. Vignettes 'Processing HapMap III reference data for ancestry estimation' and 'Processing 1000Genomes reference data for ancestry estimation', show the download and processing of the HapMap phase III and 1000Genomes phase III dataset, respectively. In this example, we will use the 1000Genomes data as the reference dataset. We recommend using `check_relatedness` on the reference to filter our related samples. This prevents data leakage between the training and testing datasets while training.

Set-up

We will first set up some bash variables and create directories needed; storing the names and directories of the reference and study will make it easy to use updated versions of the reference or new datasets in the future. We create a directory named 'qcdir' for the study data. In order to keep the data directory tidy, we'll create a directory for the log files and move them to the log directory here after each analysis step. This is useful to keep the PLINK log-files for future reference. Once you have created 'qcdir', be sure to copy the data.bim, data.bed, and data.fam files into your directory. If you are using HapMap III reference data, be sure to use the correct variable name; refname='HapMapIII'.

```
qcdir=~/qcdir
refdir=~/reference
name='data'
refname='all_hg38'

mkdir -p $qcdir/plink_log
```

Match study genotypes and reference data

In order to compute joint principal components of the reference and study population, we will need to combine the two datasets. The plink –merge function enables this merge, but requires the variants in the datasets to be matching by chromosome, position and alleles. The following sections show how to extract the relevant data from the reference and study dataset and how to filter matching variants.

Filter reference and study data for non A-T or G-C SNPs

We will use an awk script to find non-A/T and non-G/C SNPs, as these SNPs are more difficult to align, and remove them from both the reference and study data sets. In addition, we will only keep the autosomes (chr 1-22), only keep snps (snps-only), of those, keep only the biallelic ones (max-alleles 2), and remove any duplicates (rm-dup). The data should start off in Plink 1.9 data format.

```
awk 'BEGIN {OFS="\t"}  ($5$6 == "GC" || $5$6 == "CG" \
                        || $5$6 == "AT" || $5$6 == "TA")  {print $2}' \
    $qcdir/$name.bim  > \
    $qcdir/$name.ac_gt_snps
awk 'BEGIN {OFS="\t"}  ($5$6 == "GC" || $5$6 == "CG" \
                        || $5$6 == "AT" || $5$6 == "TA")  {print $2}' \
    $refdir/$refname.bim  > \
    $qcdir/$refname.ac_gt_snps

plink2 --bfile  $refdir/$refname \
      --rm-dup exclude-all \
      --max-alleles 2 \
      --snps-only just-acgt \
      --exclude $qcdir/$refname.ac_gt_snps \
      --chr 1-22 \
      --make-bed \
      --out $qcdir/$refname.no_ac_gt_snps
mv  $qcdir/$refname.no_ac_gt_snps.log $qcdir/plink_log/$refname.no_ac_gt_snps.log

plink2 --bfile  $qcdir/$name \
      --rm-dup exclude-all \
      --max-alleles 2 \
      --snps-only just-acgt \
      --exclude $qcdir/$name.ac_gt_snps \
      --make-bed \
      --chr 1-22 \
      --allow-extra-chr \
      --out $qcdir/$name.no_ac_gt_snps
mv  $qcdir/$name.no_ac_gt_snps.log $qcdir/plink_log/$name.no_ac_gt_snps.log
```

Renaming variant identifiers

Variant identifiers can vary between studies. To ensure that the data can be properly processed, we will rename the variant identifiers to a common scheme based on chromosome number and basepair location by using plinkQC's rename variant identifier function.

```
library(plinkQC)
name <- "data.no_ac_gt_snps"
```

```
refname <- "all_hg38.no_ac_gt_snps"
path2plink2 <- "/Users/syed/bin/plink2"
rename_variant_identifiers(indir=qcdir, qcdir=qcdir, name=name,
                           path2plink2 = path2plink2)
```

Filtering out the shared SNPs between the study dataset and reference 1000G dataset

```
cd $qcdir
awk '{print $3}' $name.renamed.pvar > $name.renamed.varids.txt

plink2 --pfile $refname.renamed --extract $name.renamed.varids.txt --make-bed --out $refname.renamed.stu
```

Conducting markerQC, pruning LD, and individual QC

After filtering for SNPS shared in the study, we will conduct marker and sample quality control on the reference dataset. This is to ensure only robust data is used as a reference. To reduce correlations between SNPs, we will prune for variants in linkage disequilibrium (LD) after removing low quality markers. This is done with plinkQC's pruning_ld() function. As part of the function, ranges of known high-LD are excluded. These ranges are originally provided by [1].

```
refname <- "all_hg38.renamed.studysnps"

fail_markers <- perMarkerQC(indir=indir, qcdir=qcdir, name=name,
                            path2plink=path2plink,
                            verbose=TRUE, interactive=TRUE,
                            showPlinkOutput=FALSE)
marker_ids <- cleanData(indir = indir, qcdir = qcdir, name = name,
                  path2plink = path2plink, filterAncestry = FALSE, filterRelated = FALSE, macTh = NULL, ma
                  filterSex = FALSE, filterHeterozygosity = FALSE,
                  filterSNPMissingness = TRUE, filterSampleMissingness = FALSE,
                  filterMAF = TRUE, filterHWE = TRUE)
name = paste(name, ".clean", sep = "")

pruning_ld(indir = indir, qcdir = qcdir, name = name,
      path2plink = path2plink, genomebuild="hg38")
name = paste(name, ".pruned", sep = "")

fail_samples <- perIndividualQC(indir=indir, qcdir=qcdir, name=name, dont.check_sex = TRUE,
                                dont.check_relatedness = TRUE,
                            path2plink=path2plink, dont.check_ancestry = TRUE,
                            interactive=TRUE, verbose=TRUE)

sample_ids <- cleanData(indir = indir, qcdir = qcdir, name = name,
             path2plink = path2plink, filterAncestry = FALSE, filterRelated = FALSE,verbose = TRUE,
            filterSex = FALSE, filterHeterozygosity = TRUE,
            filterSNPMissingness = FALSE, filterSampleMissingness = TRUE,
            filterMAF = FALSE, filterHWE = FALSE)
```

PCA

After conducting quality control, we will extract the principal components from the data to reduce the input space.

```
plink2 --bfile  $refname.renamed.studysnps.cleaned.pruned.cleaned \
       --nonfounders \
       --freq counts \
       --pca allele-wts 20 \
       --out $refname.renamed.studysnps.cleaned.pruned.cleaned.pca

mv $qcdir/$refname.renamed.studysnps.cleaned.pruned.cleaned.pca $qcdir/plink_log
```

After running a PCA, we will project the original dataset onto the principal components to rescale. This ensures that future projections will be in the same scale.

```
plink2 --pfile $refname.renamed.studysnps.cleaned.pruned.cleaned
       --read-freq $refname.renamed.studysnps.cleaned.pruned.cleaned.pca.acount
       --score $refname.renamed.studysnps.cleaned.pruned.cleaned.pca.eigenvec.allele 2 6 header-read no
       --score-col-nums 7-26 --out $refname.renamed.studysnps.cleaned.pruned.cleaned.projection
```

Training random forest classifier in R

The principal components of the reference dataset will be used to train the random forest algorithm in R.

```
library(tidyverse)
library(randomForest)

filepath <- 'insert path to sscore file here'
proj <- read.csv(
  file= filepath,
  sep='\t', header = TRUE)

package.dir <- find.package('plinkQC')
ancestry_info <-
  read_delim(file.path(package.dir,"extdata/Genomes1000_ID2Pop.txt"))
superpop <-
  read_delim(file.path(package.dir,"extdata/AncestryGeoLocations.csv"))
```

We divide up the data into a 70% training portion and 30% testing portion. To ensure a balanced model, we will sample the data to ensure an even representation of each ancestral superpopulation group in the testing portion of the data. The number of inidividuals from each group that is chosen will have to adjusted for the size of the reference dataset. For the 1000 genomes dataset, we use 448 samples in each of the five ancestral groups for a roughly 70% of the data.

```
proj <- proj %>%
  select(-c(ALLELE_CT, NAMED_ALLELE_DOSAGE_SUM))
colnames(proj) <- c("IID", paste0("PC", 1:20))

labeled_proj <- merge(proj, superpop)

n_individuals <- 448
set.seed(123)
idx <- split(seq(nrow(labeled_proj)), labeled_proj$Ancestry)

train.ids <- lapply(idx, function(i) {
  sample(i, n_individuals)
})

ids_to_keeps<- unlist(train.ids, use.names = FALSE, recursive = FALSE)
train_proj_1000g <- labeled_proj[ids_to_keeps,]
```

Then, we can train the random forest. We are putting in a starting value for the parameter but we recommend utilizing a grid search (described in detail more below) to determine the optimal values.

```
train_proj_1000g$Ancestry <- factor(train_proj_1000g$Ancestry)

ancestry_rf <- randomForest(Ancestry ~ .,
                        data = train_proj_1000g[,-c(2,23)],
                        method = "rf",
                        ntree = 750,
                        importance = TRUE)
ancestry_rf
```

Predicting ancestries of new study data

To predict the ancestries of a new study dataset with our trained random forest, you need to project the newdata onto the principal components of the reference dataset. This is done with the –score function in plink2. The projection is then used as an input for the random forest.

```
plink2 --pfile newdata
       --read-freq all_hg38.pca.acount
       --score all_hg38.pca.eigenvec.allele 2 6 header-read no-mean-imputation variance-standardize
       --score-col-nums 7-26 --out newdata.projection
```

Then, we can use the random forest to predict the ancestry of the newdata.

```
filepath <- "Insert path to newdata.sscore here"
newdata <- read.csv(
  file= filepath,
  sep='\t', header = TRUE)

newdata <- newdata %>%
  select(-c(ALLELE_CT, NAMED_ALLELE_DOSAGE_SUM))
colnames(newdata) <- c("ID", paste0("PC", 1:20))

predictions <- predict(ancestry_rf, newdata)
```

Evalulating and Tuning Classification

To evaluate the accuracy of the model, a commonly used metric is the out-of-bag error rate and confusion matrix. The confusion matrix displays the number of correct classifications for each ancestry along the diagonal. Any values outside the diagonal are misclassifications for that other ancestry. A summary of these metrics can be seen through:

```
ancestry_rf
```

And we can visualize the confusion matrix as a heatmap.

```
my_rf <- ancestry_rf$confusion
heatmap(my_rf[,-c(27)], scale = 'column', trace = 'none')
```

Parameter Tuning

Tuning the parameters for this random forest is crucial in building a machine-learning model to produce the best forest. In this case, having the highest accuracy.

Grid Search

There are different optimization techniques to determine the parameters for a random forest. A simple one to implement is a grid-search in where models with different parameters are initialized. Based on their performance, the optimal parameters can be determined. The parameters tested in a grid search are the number of principal components included and the number of trees. Within the parameter values of the number of principal components and number of trees, cross-validation is done to determine the mtry variable.

```r
train_results <- data.frame(mtry = c(0), ntrees = c(0), num_pc = c(0), acc = c(0))
train_proj_noids <- train_proj_1000g[,-c(1)]
for (PC_inc in c(1:20)) {
  for (ntree in c(1, 5)) {
    set.seed(123)
    fit <- train(Pop~., data=train_proj_noids[,c(1:PC_inc,21)],
                 method="rf", metric="Accuracy",
                 trControl=control, ntree=ntree)
    train_proj_1000g <- rbind(train_proj_1000g,
                      data.frame(mtry = fit$results$mtry,
                                 ntrees = rep(ntree, nrow(fit$results)),
                                 num_pc = rep(PC_inc, nrow(fit$results)),
                                 acc = fit$results$Accuracy))
  }
}
```

Evaluating/Interpretting the RF

Once you have found a successful OOB error rate, you can use the below package to find out important information regarding your tree. The below documentation builds a decision tree using categorical aspects to classify the data. The PC values for each sample are provided in our 1000 Genomes reference dataset file, where each sample has values for 20 distinct PCs. We ask if a certain sample's value for a specific PC is less than or greater than a given number. Based on that answer, it is either classified as a specific ancestry or needs to undergo more investigation to classify it correctly. For comprehensability, this is only a subset of a decision tree the random forest builds as classifying all 26 ancestries builds quite a complicated and cluttered plot.

```r
# This is a visual of the random forest being made.
# https://github.com/araastat/reprtree
reprtree:::plot.getTree(rf_mtry)
# Two different plots
reprtree:::plot.getTree(rf_mtry, k=1, d=5)
reprtree:::plot.getTree(rf_mtry, k=1, d=6)
```

References

1. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nature Protocols. 2010;5: 1564–73. doi:10.1038/nprot.2010.116