FAUST PFDA

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This vignette provides a demonstration of how to apply PFDA to the annotated count matrix produced by FAUST. It is a mechanical demonstration, insofar as we will simulate data and show the steps involved in fitting a PFDA model. In real data, care must be taken to properly define the biological compartment of interest in terms of the marker panel prior to fitting the PFDA model.

PFDA example

We begin by simulating a dataset.

```
#
#generate the annotation thresholds
#
set.seed(123)
projPath <- file.path(tempdir(),"FAUST")
if (dir.exists(projPath)) {
   unlink(projPath, recursive=TRUE)
}
dir.create(projPath, recursive = TRUE)
faust(
    gatingSet           = gs,
    startingCellPop     = "root",
    projectPath         = projPath,
    plottingDevice      = "png",
    annotationsApproved = TRUE,
    threadNum           = 4
)
```

We next load the counts into the analysis and derive a parent count. To demonstrate fitting a PFDA model, we will manually assign a reponder status to each sample against which we can model.

```
count_df <- as.data.frame(readRDS(file.path(projPath,"faustData","faustCountMatrix.rds")))
cellPops <- setdiff(colnames(count_df),c("0_0_0_0_0"))
count_df$parentCount <- apply(count_df,1,sum)
count_df$subjectID <- paste0("Sample_",sprintf('%0.2d', 1:nrow(count_df)))
count_df$modelRT <- c(rep(1,(nrow(count_df)/2)),rep(0,(nrow(count_df)/2)))
```

To apply PFDA, we must have a biological compartment in mind whose sub-phenotypes we wish to investigate. Here we are simulating data, and so will assume phenotypes that are `V1+` represent a biological compartment of interest in order to demonstrate the process.

```
pfdaExampleCellPops <- cellPops[grepl("V1\\+",cellPops)]
pfdaExampleGlmerDFPrep <- count_df[,c(pfdaExampleCellPops,"parentCount","modelRT","subjectID")]
pfdaExampleGlmerDF <- gather(pfdaExampleGlmerDFPrep,key=cellPop,value=count,-
c("parentCount","modelRT","subjectID"))
pfdaExampleGlmerDF$popName <-
as.factor(paste0("Pop_",as.numeric(as.factor(pfdaExampleGlmerDF$cellPop))))
```

```
pfdaExampleGlmerDF$obsFactor <- as.factor(paste0("Obs_",seq(nrow(pfdaExampleGlmerDF))))
kable(pfdaExampleCellPops)
```

|x | |:----------------------| |V4-V3-V8-V6+V5-V7+V2-V1+ | |V4-V3-V8-V6+V5+V7+V2+V1+ | |V4-V3-V8+V6-V5-V7-V2+V1+ | |V4-V3+V8-V6-V5-V7+V2-V1+ | |V4-V3+V8+V6-V5+V7-V2-V1+ | |V4-V3+V8+V6-V5+V7+V2-V1+ | |V4-V3+V8+V6+V5-V7-V2-V1+ | |V4+V3-V8-V6-V5+V7-V2+V1+ | |V4+V3-V8-V6-V5+V7+V2-V1+ | |V4+V3-V8+V6-V5-V7+V2-V1+ | |V4+V3-V8+V6+V5-V7+V2+V1+ | |V4+V3-V8+V6+V5+V7+V2-V1+ | |V4+V3+V8-V6-V5-V7+V2+V1+ | |V4+V3+V8+V6-V5-V7-V2+V1+ | |V4+V3+V8+V6-V5-V7+V2-V1+ |

We now fit the multivariate PFDA model to the listed phenotypes in the compartment using a binomial GLMM using `glmer` from the `lme4` package.

```
pfdaExampleGlmerFit <- glmer(
    formula=cbind(count,(parentCount-count))  ~ popName*modelRT + (1|obsFactor),
    data=pfdaExampleGlmerDF,
    family="binomial",
    nAGQ=0,
    control=glmerControl(
        optimizer="bobyqa",
        optCtrl = list(maxfun = 1e9),
        boundary.tol = 1e-9,
        tolPwrss=1e-9,
        check.conv.singular = .makeCC(action = "warning",  tol = 1e-12)
    )
)
```

```
#> Error in pwrssUpdate(pp, resp, tol = tolPwrss, GQmat = GHrule(0L), compDev = compDev, :
Downdated VtV is not positive definite
```

Having fit the model, we conduct a test of increased abundance across the compartment using `glht` from the `multcomp` package.

```
pfdaExampleCoefNames = names(fixef(pfdaExampleGlmerFit))
```

```
#> Error in fixef(pfdaExampleGlmerFit): object 'pfdaExampleGlmerFit' not found
```

```
pfdaExampleGLHTMat <- matrix(0, nrow = 1, ncol = length(pfdaExampleCoefNames))
```

```
#> Error in matrix(0, nrow = 1, ncol = length(pfdaExampleCoefNames)): object
'pfdaExampleCoefNames' not found
```

```
pfdaExampleGLHTMat[,which(pfdaExampleCoefNames == "modelRT")] <- 1
```

```
#> Error in pfdaExampleGLHTMat[, which(pfdaExampleCoefNames == "modelRT")] <- 1: object
'pfdaExampleGLHTMat' not found
```

```
pfdaExampleGLHTMat[1,which(grepl(":modelRT",pfdaExampleCoefNames))] <-
1/(length(which(grepl(":modelRT",pfdaExampleCoefNames)))+1)
```

```
#> Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method
for function 'which': object 'pfdaExampleCoefNames' not found
```

```
rownames(pfdaExampleGLHTMat) <- c("Phenotype_Compartment")
```

```
#> Error in rownames(pfdaExampleGLHTMat) <- c("Phenotype_Compartment"): object
'pfdaExampleGLHTMat' not found
```

```
pfdaExampleMulti <- glht(pfdaExampleGlmerFit,pfdaExampleGLHTMat, alternative = "greater")
```

```
#> Error in glht(pfdaExampleGlmerFit, pfdaExampleGLHTMat, alternative = "greater"): object
'pfdaExampleGLHTMat' not found
```

```
pfdaExampleObsPval <- round(summary(pfdaExampleMulti)$test$pvalues[1],9)
```

```
#> Error in h(simpleError(msg, call)): error in evaluating the argument 'object' in selecting a
method for function 'summary': object 'pfdaExampleMulti' not found
```

```
pfdaExampleCT95 <- confint(pfdaExampleMulti,level=0.95)
```

```
#> Error in confint(pfdaExampleMulti, level = 0.95): object 'pfdaExampleMulti' not found
```

```
pfdaExampleMultiResults <- as.data.frame(pfdaExampleCT95$confint)
```

```
#> Error in as.data.frame(pfdaExampleCT95$confint): object 'pfdaExampleCT95' not found
```

```
pfdaExampleMultiResults$Compartment <- rownames(pfdaExampleMultiResults)
```

```
#> Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method
for function 'rownames': object 'pfdaExampleMultiResults' not found
```

```
pfdaExampleMultiResults$ModelType <- "PFDA"
```

```
#> Error in pfdaExampleMultiResults$ModelType <- "PFDA": object 'pfdaExampleMultiResults' not
found
```

```
pfdaExampleMultiResults$Component <- "PFDA"
```

```
#> Error in pfdaExampleMultiResults$Component <- "PFDA": object 'pfdaExampleMultiResults' not
found
```

```
kable(pfdaExampleMultiResults)
```

```
#> Error in kable(pfdaExampleMultiResults): object 'pfdaExampleMultiResults' not found
```

This demonstrates the mechanics of how to run `PFDA` on the `FAUST` count matrix.