uneven\_timepoints\_hourly.R

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library(sleuth, splines)  
library(biomaRt)

## Warning: replacing previous import 'utils::findMatches' by  
## 'S4Vectors::findMatches' when loading 'AnnotationDbi'

ensembl <- biomaRt::useEnsembl(biomart = "genes",  
 dataset = "hsapiens\_gene\_ensembl",  
 mirror='useast')  
t2g <- biomaRt::getBM(attributes = c("ensembl\_transcript\_id",   
 "ensembl\_gene\_id",  
 "external\_gene\_name"),  
 mart = ensembl)  
t2g <- dplyr::rename(t2g, target\_id = ensembl\_transcript\_id,  
 ens\_gene = ensembl\_gene\_id,  
 ext\_gene = external\_gene\_name)  
  
setwd("~/longsaliva")  
s2c <- read.delim("s2c\_hourly.txt", sep=" ", header=TRUE)  
time <- rep(seq(from=1, to=length(s2c$sample)/2, by=1), times=2)  
s2c <- dplyr::mutate(s2c, time=time)  
sample <- paste0(rep(c('pre', 'post'), each=20),'\_',s2c$time)  
s2c$sample <- sample  
  
colnames(s2c) <- c("path", "sample", "condition", "time")  
# If the time points between pre- and post- are matched  
# s2c <- s2c[c(1, 2, 4, 8, 20, 21, 22, 24, 28, 40), ]  
  
# This works when time points aren't matched too  
s2c <- s2c[c(1, 2, 4, 8, 20, 21, 23, 26, 31, 40), ]  
  
group <- relevel(factor(s2c$condition), ref='pre\_vaccination')  
  
so <- sleuth\_prep(s2c, target\_mapping = t2g,  
 aggregation\_column = "ens\_gene",  
 extra\_bootstrap\_summary = TRUE)

## Warning in check\_num\_cores(num\_cores): It appears that you are running Sleuth from within Rstudio.  
## Because of concerns with forking processes from a GUI, 'num\_cores' is being set to 1.  
## If you wish to take advantage of multiple cores, please consider running sleuth from the command line.

## reading in kallisto results

## dropping unused factor levels

## ..........

## Warning in check\_target\_mapping(tmp\_names, target\_mapping,  
## !is.null(aggregation\_column)): intersection between target\_id from kallisto  
## runs and the target\_mapping is empty. attempted to fix problem by removing .N  
## from target\_id, then merging back into target\_mapping. please check  
## obj$target\_mapping to ensure this new mapping is correct.

## normalizing est\_counts  
## 25499 targets passed the filter  
## normalizing tpm  
## merging in metadata  
## summarizing bootstraps  
## ..........

X <- splines::ns(s2c$time, df=3)  
full\_design <- model.matrix(formula(~0 + group + group:X))  
colnames(full\_design)

## [1] "grouppre\_vaccination" "grouppost\_vaccination"   
## [3] "grouppre\_vaccination:X1" "grouppost\_vaccination:X1"  
## [5] "grouppre\_vaccination:X2" "grouppost\_vaccination:X2"  
## [7] "grouppre\_vaccination:X3" "grouppost\_vaccination:X3"

so <- sleuth\_fit(so, full\_design, "full")

## fitting measurement error models  
## shrinkage estimation  
## 2 NA values were found during variance shrinkage estimation due to mean observation values outside of the range used for the LOESS fit.  
## The LOESS fit will be repeated using exact computation of the fitted surface to extrapolate the missing values.  
## These are the target ids with NA values: ENST00000361624.2, ENST00000633925.1  
## computing variance of betas

so <- sleuth\_fit(so, ~0+group, "reduced")

## fitting measurement error models  
## shrinkage estimation  
## 11 NA values were found during variance shrinkage estimation due to mean observation values outside of the range used for the LOESS fit.  
## The LOESS fit will be repeated using exact computation of the fitted surface to extrapolate the missing values.  
## These are the target ids with NA values: ENST00000415137.6, ENST00000455520.6, ENST00000460988.1, ENST00000470365.5, ENST00000477261.1, ENST00000486992.5, ENST00000489840.1, ENST00000495238.1, ENST00000496209.1, ENST00000617859.4, ENST00000633925.1  
## computing variance of betas

so <- sleuth\_lrt(so, "reduced", "full")  
  
sleuth\_table <- sleuth\_results(so, 'reduced:full', 'lrt', show\_all = FALSE, pval\_aggregate = TRUE)  
sleuth\_de <- dplyr::filter(sleuth\_table, qval <= 0.05)  
head(sleuth\_de)

## target\_id ext\_gene num\_aggregated\_transcripts sum\_mean\_obs\_counts  
## 1 ENSG00000168010 ATG16L2 14 64.90701  
## 2 ENSG00000105835 NAMPT 33 178.67605  
## 3 ENSG00000172354 GNB2 10 47.56590  
## 4 ENSG00000141480 ARRB2 15 75.94359  
## 5 ENSG00000003402 CFLAR 15 62.18524  
## 6 ENSG00000143226 FCGR2A 14 68.59125  
## pval qval  
## 1 3.802128e-17 4.789541e-13  
## 2 4.176548e-16 2.630599e-12  
## 3 1.380152e-15 5.245765e-12  
## 4 1.945904e-15 5.245765e-12  
## 5 2.498578e-15 5.245765e-12  
## 6 2.453743e-15 5.245765e-12