Isoform Switch Analysis Exercise

Part 1 : An introduction

Make sure you have installed IsoformSwitchAnalyzeR

- library(IsoformSwitchAnalyzeR)
- If not you need to follow the installation instructions on Absalon

Overal Exercise Idea

- Aim:
  Get familiar with the overall workflow of IsoformSwitchAnalyzeR
- Approach:
  Use the vignette of IsoformSwitchAnalyzeR and answer questions along the way.

Assignment

Individually read/work through the following sections of the vignette:

- “Abstract”
- “Workflow Overview”
- “Short Example Workflow” - in this section you should also run the R code and check in/ output

And answer the questions in the rests of the slides. Remember to look up the documentation of the functions (run ?functionName) as all the details are there.

Open vignette

Load library into R:
library(IsoformSwitchAnalyzeR)

Open vignette

vignette("IsoformSwitchAnalyzeR")

Online alternative here

Questions

- Q1: What is the core functionalities of IsoformSwitchAnalyzeR?
- Q2: What external sequence analysis tools are currently supported and what do they do?

Questions

- Q3: Why is it smart to identify isoform switches as the first step in a workflow? (hint look at the documentation of isoformSwitchAnalysisPart1())
- Q4: How many high-level functions (functions that automatically performs multiple step of the pipeline) are there in IsoformSwitchAnalyzeR?

Questions

- Q5: What is a BSgenome object and why is it needed
- Q6: What is the result if you change the dIFcutoff parameter to 0.5 in the isoformSwitchAnalysisPart1()

Questions

- Q7: What is the main functionality of isoformSwitchAnalysisPart1() vs isoformSwitchAnalysisPart2()
- Q8: How many switches with consequences did you identify (when using a dIF cutoff as 0.5):

Questions

- Q9: What is the consequence of the switch in the “LDLRAD2” gene? (identify the predicted functional consequence)
- Q10: Why is a cutoff on both the q-value (alpha) and the dIF necessary?

Questions

- Q11: How many genes have an isoform switch in both conditions of the “exampleSwitchListAnalyzed” data?
- Q12: Use the extractConsequenceEnrichment to figure out which enrichment/depletion is the most certain (smallest q-value)

Questions

- Q13*: Using a alpha of 0.05 answer: For many genes with isoform switch are the gene also differentially expressed and what does that indicate?

Isoform Switch Analysis Exercise

Part 2: Improved understanding

Overal Exercise Idea

- Aim:
  To dig into the details of an IsoformSwitchAnalyzeR workflow
- Approach:
  Use the IsoformSwitchAnalyzeR vignette and answer questions along the way.

Assignment

Thoroughly read/work (meaning run the R code and check output) through the following sections of the vignette:

- “Detailed Workflow”

And answer the questions in the rests of the slides.

Remember to look up the function documentation for all functions you use as all the details are there.

Questions

- Q1: How many functions are isoformSwitchAnalysisPart1() and isoformSwitchAnalysisPart2() internally using?
- Q2: What class is the switchAnalyzeRlist object?
- Q3: What is the name of the main entry in the switchAnalyzeRlist object, how is it accessed and what does each row correspond to?

Questions

- Q4: What does all the analyze* and extract* functions do?
- Q5: Which functions can be ude to importing RNA-seq quantification data and creating a switchAnalyzeRlist?

Questions

- Q6: Why is the summary statistics of these two commands not different:
  e1 <- preFilter(exampleSwitchListAnalyzed, keepIsoformInAllConditions = TRUE)
  e2 <- preFilter(exampleSwitchListAnalyzed, keepIsoformInAllConditions = FALSE)

  When the resulting switchAnalyzeRlists are different:
  > nrow(e1) == nrow(e2)
  [1] FALSE

- Q7: How many different ways of testing for isoform switches are supported by IsoformSwitchAnalyzeR and which is the (current) recommended?

- Q8*: Which algorithms for identifying ORFs are supported? Which is the default and what do you think of that?

Questions

- Q9: Which consequences are affected by the ‘ntCutoff’ and the ‘AaJCsimCutoff’ partner in the analysis of switch consequences, and what does the cutoffs do.

- Q10: How many different types of consequences can be predicted from the domain annotation and why is there a need for all of them?

- Q11: How do you access the details of the consequence analysis and which is the first two isoforms compared in the example (use exampleSwitchListAnalyzed)?

Questions

- Q12*: Using exampleSwitchListAnalyzed: What is the overlap between the top 10 switches (with consequences) and what does that indicate.
- Q13: What is the consequence of the switch in the gene which is nr 3 on the top list when sorting by Values values?
- Q14: What is the relation between isoform switches and gene log2 FC?

Questions

- Q15*: Visually inspect the relation between isoform switches and gene expression