This document is a work in progress and will continually be updated as new features or analyses are integrated into the cummeRbund pipeline. This guide is being released as is, with the understanding that existing gaps will be completed in due time. Please bear with us as we work to expand this resource.

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1 Overview

2 Workflow Summary

3 Reading cuffdiff output

> library(cummeRbund)

> cuff <- readCufflinks(dir=system.file("extdata", package="cummeRbund"))
> cuff

CuffSet instance with:
   3 samples
   400 genes
   1203 isoforms
   662 TSS
   906 CDS
   1062 promoters
   1986 splicing
   990 relCDS

This example uses the ‘test’ dataset that is included in the cummeRbund package. Importantly, if your current working directory contains the output from your cuffdiff analysis, simply calling cuff<-readCufflinks() will automatically find the default files and begin parsing your data.

4 Quality Assessment of data

4.1 Evaluating model fit

> d<-dispersionPlot(genes(cuff))
> d
4.2 Identifying outlier replicates

> pBoxRep<-csBoxplot(genes(cuff),replicates=T)
> pBoxRep

> pDendro<-csDendro(genes(cuff),replicates=T)
> pDendro

'dendrogram' with 2 branches and 6 members total, at height 0.2685017
A dendrogram with 2 branches and 6 members total, at height 0.2685017
4.3 Determining relationships between conditions

> pBox<-csBoxplot(genes(cuff))
> pBox
5 Analysis of differential expression

5.1 Identifying differentially expressed genes

> sigGeneIds <- getSig(cuff, alpha=0.05, level="genes")
> head(sigGeneIds)

[1] "XLOC_000004" "XLOC_000005" "XLOC_000008" "XLOC_000009"
[5] "XLOC_000011" "XLOC_000013"

> length(sigGeneIds)

[1] 207

> hESCvsFibroblast.sigGeneIds <- getSig(cuff, "hESC","Fibroblasts",alpha=0.05,level="genes")
> head(hESCvsFibroblast.sigGeneIds)

[1] "XLOC_000005" "XLOC_000009" "XLOC_000013" "XLOC_000014"
[5] "XLOC_000018" "XLOC_000025"

> length(hESCvsFibroblast.sigGeneIds)

[1] 151
5.1.1 Creating significant gene sets

> sigGenes<-getGenes(cuff,sigGeneIds)
> sigGenes

CuffGeneSet instance for 207 genes

Slots:
  annotation
  fpkm
  repFpkm
  diff
  count
  isoforms  CuffFeatureSet instance of size 717
  TSS        CuffFeatureSet instance of size 399
  CDS        CuffFeatureSet instance of size 577
  promoters  CuffFeatureSet instance of size 207
  splicing   CuffFeatureSet instance of size 399
  relCDS     CuffFeatureSet instance of size 207

5.1.2 Visualization of significant gene sets

5.2 Identifying differentially expressed features

> sigGeneIds<-getSig(cuff,alpha=0.05,level="isoforms")
> head(sigGeneIds)

[1] "TCONS_00000007" "TCONS_00000008" "TCONS_00000021"
[4] "TCONS_00000022" "TCONS_00000033" "TCONS_00000041"

> length(sigGeneIds)
[1] 256

5.2.1 Creating significant feature sets

5.2.2 Visualization of significant feature sets

5.3 Analysis of individual genes

5.3.1 Subsetting individual genes

5.3.2 Creating a CuffGene object

> Pink1<-getGene(cuff,'PINK1')
> Pink1

CuffGene instance for gene XLOC_000172
Short name: PINK1
Slots:
5.3.3 Visualization of individual genes

6 Data Exploration

6.1 Identifying patterns of gene expression
Partitioning around medoids as e.g.

6.2 Similarity analysis
findSimilar

6.3 Specificity analysis
csSpecificity

7 Analysis of differential splicing

8 Analysis of differential promoter usage

9 Linking with additional analyses

9.1 Gene Ontology Analysis (clusterProfiler)
In this particular example, the reference .gtf file that was used has the official gene symbol (HUGO) stored in the gene name attribute field. This value is carried over by cuffdiff and cummeRbund into the gene_short_name field for each feature. We will exploit this ID and use it to map genes to their Entrez ID to be able to use the very nice clusterProfiler package.

The first step is to translate

9.2 Gene Set Enrichment Analysis (Preranked)
We will generate .rnk and .gmx files for use in the GSEA Preranked analysis.
9.3 Principal component analysis

10 Visualization Tips/Tricks

10.1 Faceting

11 Session Info