CummeRbund workflow

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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)</pre>

```
> pDendro
```

<code>'dendrogram'</code> with 2 branches and 6 members total, at height 0.2685017



'dendrogram' with 2 branches and 6 members total, at height 0.2685017 'dendrogram' with 2 branches and 6 members total, at height 0.2685017

All genes(cuff)



Determining relationships between conditions **4.3**

> 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)</pre> > sigGenes CuffGeneSet instance for 207 genes Slots: annotation fpkmrepFpkm diff count CuffFeatureSet instance of size 717 isoforms TSS CuffFeatureSet instance of size 399 CDS CuffFeatureSet instance of size 577 CuffFeatureSet instance of size 207 promoters 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")</pre>
> 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
     Analysis of individual genes
5.3
5.3.1 Subsetting individual genes
5.3.2 Creating a CuffGene object
> Pink1<-getGene(cuff, 'PINK1')</pre>
> Pink1
CuffGene instance for gene XLOC_000172
Short name:
                   PINK1
Slots:
```

```
annotation

features

fpkm

repFpkm

diff

count

isoforms

CuffFeature instance of size 2

TSS

CuffFeature instance of size 2

CDS

CuffFeature instance of size 2
```

5.3.3 Visualization of individual genes

6 Data Exploration

6.1 Identifying patterns of gene expression

Partitioning around medioids as e.g.

6.2 Similarity analysis

 $\mathit{findSimilar}$

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