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.

Contents

1 Overview

2 Workflow Summary

3 Reading cuffdiff output

```
> library(cummeRbund)
```

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

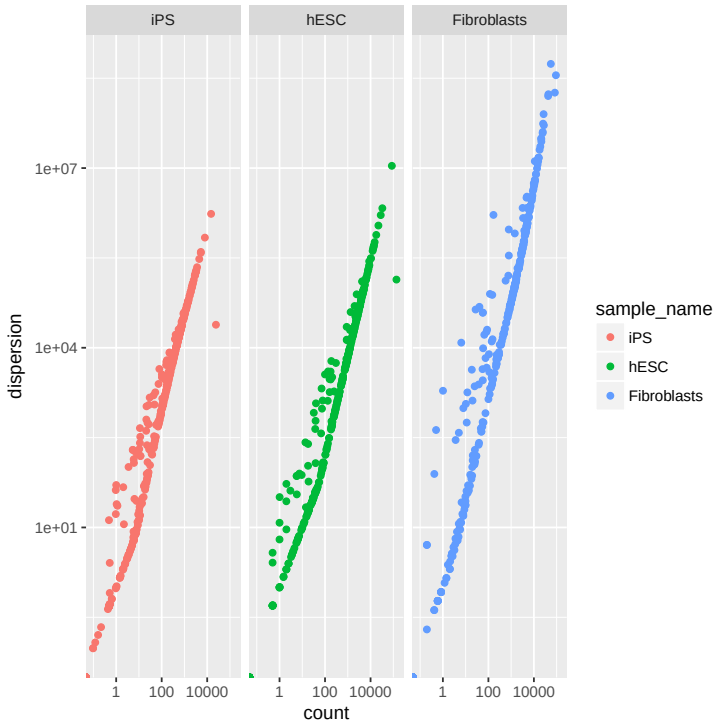
```
CuffSet instance with:
         3 samples
         400 genes
         1203 isoforms
         662 TSS
         906 CDS
         1062 promoters
         1986 splicing
         990 relCDS
```

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
```

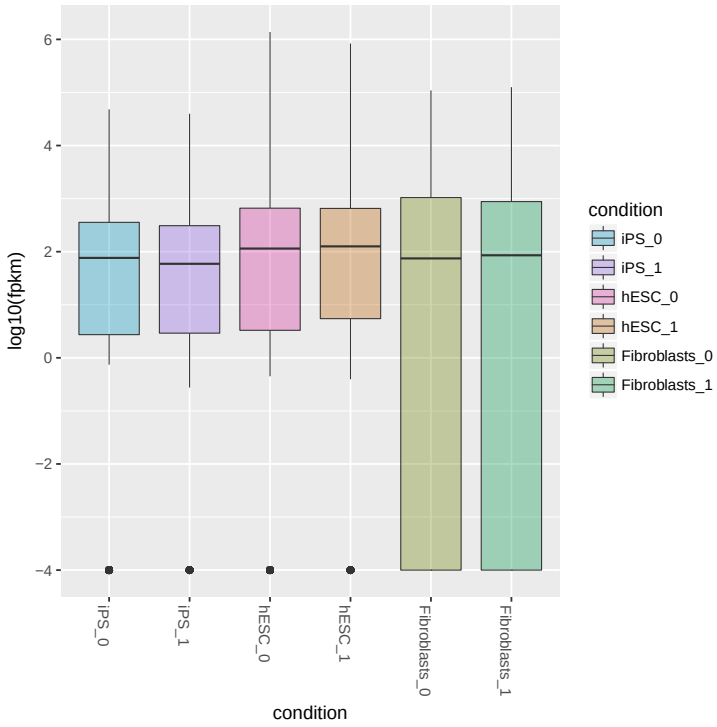


4.2 Identifying outlier replicates

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

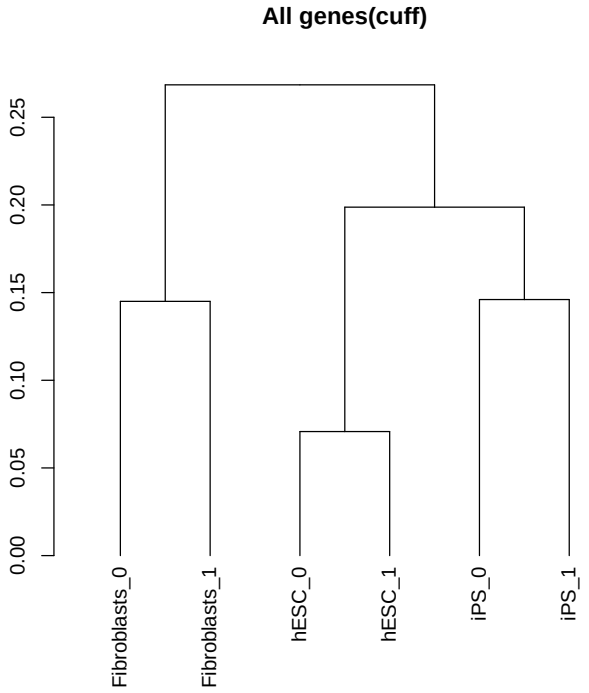
```
> pDendro<-csDendro(genes(cuff),replicates=T)
> pDendro
```

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



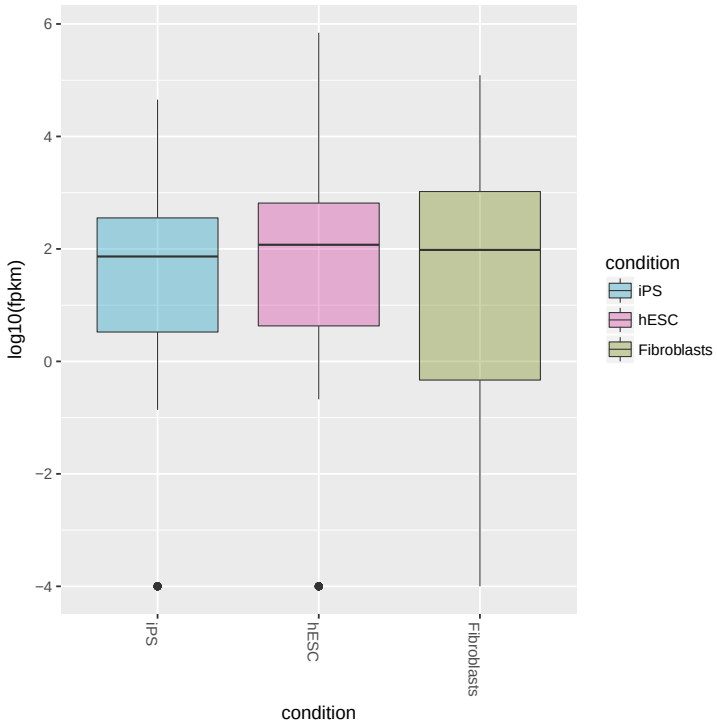

```
'dendrogram' with 2 branches and 6 members total, at height 0.2685017
```


```
'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:
```

```
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

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.