COSICC_DA_group and COSICC_DA_lineage

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This tutorial illustrates how to use COSICC_DA_group with SingleCellExperiments and with SeuratObjects.

```
knitr::opts_chunk$set(
  warning = FALSE,
  message = FALSE,
  error = FALSE
)

suppressPackageStartupMessages(library(COSICC))
```

COSICC_DA_group for SingleCellExperiments

First, we simulate a chimera-style data set, where cells have a fluorescent marker tdTomato or not.

```
sim_data_sce <- simulate_sce(seed = 10)
```

The function above simulated a knockout and a wild-type chimera data set.

```
sce_knockout <- sim_data_sce$case
sce_WT <- sim_data_sce$control
```

Let's have a look at the simulated data sets:

```
colData(sce_knockout)
```

```
## DataFrame with 3894 rows and 5 columns
##                 Cell       Batch celltype ExpLibSize    tdTomato
##          <character> <character> <factor>  <numeric> <character>
## Cell1355    Cell1355      Batch1  Group8     57934.8         pos
## Cell136      Cell136      Batch1  Group8     63484.6         pos
## Cell4675    Cell4675      Batch1  Group9     68405.4         pos
## Cell1033    Cell1033      Batch1  Group9     58145.4         pos
## Cell4670    Cell4670      Batch1  Group10    65819.3         pos
## ...              ...         ...      ...        ...         ...
## Cell1623    Cell1623      Batch1   Group4    75971.8         neg
## Cell3078    Cell3078      Batch1   Group4    60108.4         neg
## Cell943      Cell943      Batch1   Group6    57467.0         neg
## Cell4234    Cell4234      Batch1   Group4    53096.3         neg
## Cell4793    Cell4793      Batch1   Group7    55155.8         neg
```

```
colData(sce_WT)
```

```
## DataFrame with 5000 rows and 5 columns
##                 Cell       Batch celltype ExpLibSize    tdTomato
##          <character> <character> <factor>  <numeric> <character>
```

```
## Cell1          Cell1      Batch1   Group1    66621.0         neg
## Cell2          Cell2      Batch1   Group9    72881.1         pos
## Cell3          Cell3      Batch1   Group4    59400.4         neg
## Cell4          Cell4      Batch1   Group3    92392.0         pos
## Cell5          Cell5      Batch1   Group8    43452.9         neg
## ...              ...         ...      ...       ...         ...
## Cell4996    Cell4996      Batch1   Group6    56514.5         neg
## Cell4997    Cell4997      Batch1   Group5    61676.2         neg
## Cell4998    Cell4998      Batch1   Group9    30729.4         neg
## Cell4999    Cell4999      Batch1   Group8    72140.7         pos
## Cell5000    Cell5000      Batch1   Group6    57851.1         neg
```

The tdTomato coloumn contains the values “pos” and “neg”.

To use COSICC_DA_group we need to rename them to TRUE and FALSE.

```
sce_WT$tdTomato <- sce_WT$tdTomato == "pos"
sce_knockout$tdTomato <- sce_knockout$tdTomato == "pos"
```

Furthermore, we need to rename the colData.

```
names(colData(sce_WT))[names(colData(sce_WT))== "tdTomato"] <- "marked"
names(colData(sce_WT))[names(colData(sce_WT)) == "celltype"] <- "cell_type"

names(colData(sce_knockout))[names(colData(sce_knockout))== "tdTomato"] <- "marked"
names(colData(sce_knockout))[names(colData(sce_knockout)) == "celltype"] <- "cell_type"
```

We also make sure that the cells from the WT and knockout data sets have different names.

```
colnames(sce_WT) <- paste0(colnames(sce_WT),"_WT")
colnames(sce_knockout) <- paste0(colnames(sce_knockout),"_knockout")
```

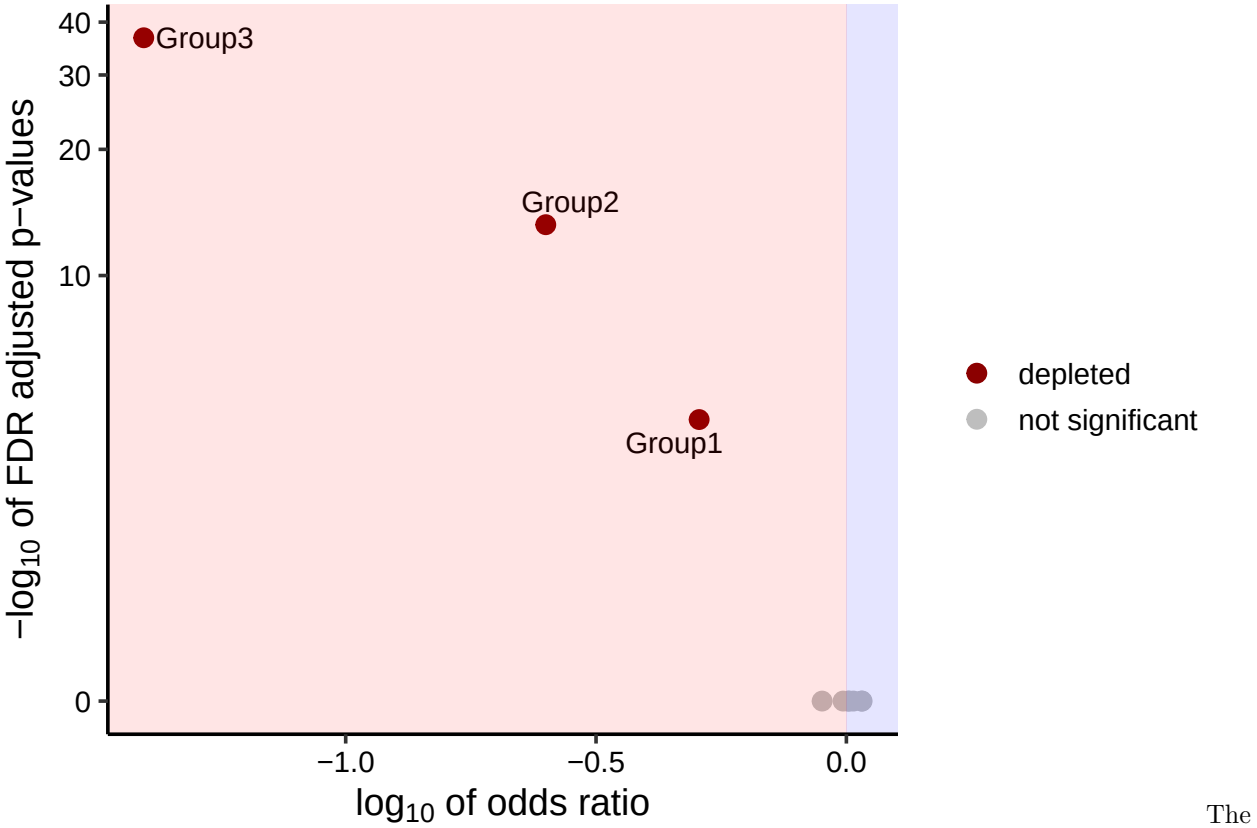
Now the data look as follows:

```
head(colData(sce_WT))
```

```
## DataFrame with 6 rows and 5 columns
##                 Cell       Batch cell_type ExpLibSize    marked
##          <character> <character>  <factor>  <numeric> <logical>
## Cell1_WT       Cell1      Batch1    Group1    66621.0     FALSE
## Cell2_WT       Cell2      Batch1    Group9    72881.1      TRUE
## Cell3_WT       Cell3      Batch1    Group4    59400.4     FALSE
## Cell4_WT       Cell4      Batch1    Group3    92392.0      TRUE
## Cell5_WT       Cell5      Batch1    Group8    43452.9     FALSE
## Cell6_WT       Cell6      Batch1    Group1    73600.7     FALSE
```

We can now COSICC_DA_group to identify depletion and/or enrichment of cell types for the tdTomato positive cells in the knockout chimeras.

```
DA_result_sce <- COSICC_DA_group(
    sce_case=sce_knockout,
    sce_control=sce_WT
)
```



The figure above illustrates the cell types Group1, Group2 and Group3 are depleted for the tdTomato positive group in the knockout data set compared to the wild-type data set.

The output of the function COSICC_DA_group is a data frame with the following columns.

Column Name	Description
`cell_type`	Cell type name.
`FDR`	FDR computed using the Benjamini-Hochberg method.
`odds_ratio`	Odds ratio of enrichment/depletion for each group of cells or cell type.
`sig`	Significance status: “enriched”, “depleted”, or “not significant”.

Below we print the output.

```
DA_result_sce
```

```
##    cell_type     p_values odds_ratio             sig
## 3     Group3 1.815058e-37 0.03950769        depleted
## 2     Group2 5.769775e-14 0.25097447        depleted
## 1     Group1 3.974107e-05 0.50807159        depleted
## 4     Group4 1.000000e+00 1.07278126 not significant
## 5     Group5 1.000000e+00 1.07482222 not significant
## 6     Group6 1.000000e+00 1.01275506 not significant
## 7     Group7 1.000000e+00 0.89440486 not significant
## 8     Group8 1.000000e+00 1.03276313 not significant
## 9     Group9 1.000000e+00 1.00808883 not significant
## 10   Group10 1.000000e+00 0.98486236 not significant
```

COSICC_DA_group for SeuratObject

First, we simulate a chimera-style data set, where cells have a fluorescent marker tdTomato or not.

```
library(Seurat)
sim_data_seurat <- simulate_seurat(seed = 10)
seurat_knockout <- sim_data_seurat$case
seurat_WT <- sim_data_seurat$control
```

Now we create SingleCellExperiments.

```
sce_WT <- SingleCellExperiment(assays=list(counts=seurat_WT@assays$RNA),colData=seurat_WT@meta.data)
sce_knockout <- SingleCellExperiment(assays=list(counts=seurat_knockout@assays$RNA),colData=seurat_knoc
```

Now you can use COSICC_DA_group as described in the section on COSICC_DA_group for SingleCellExperiments above.

COSICC_DA_lineage

To illustrate COSICC_DA_lineage, we simulate lineage scores. In application in development, these scores might be scores indicating probabilities of a cells turning into each lineage. We use example data from the package.

```
data(package="COSICC")
```

This shows that the package contains the following example data sets:

lineage_scores
sce_DA_lineage_case
sce_DA_lineage_control

Note that sce_DA_lineage_case and sce_DA_lineage_control have a slot cell in the colData. This is the cell ID, or the ID of the mapped cell in a reference atlas. It needs to be identical to the id column of the lineage_scores data frame (see below).

```
head(sce_DA_lineage_case)
```

```
## class: SingleCellExperiment
## dim: 0 300
## metadata(0):
## assays(0):
## rownames: NULL
## rowData names(0):
## colnames: NULL
## colData names(2): cell marked
## reducedDimNames(0):
## mainExpName: NULL
## altExpNames(0):
```

The scores look as follows:

```
head(lineage_scores )
```

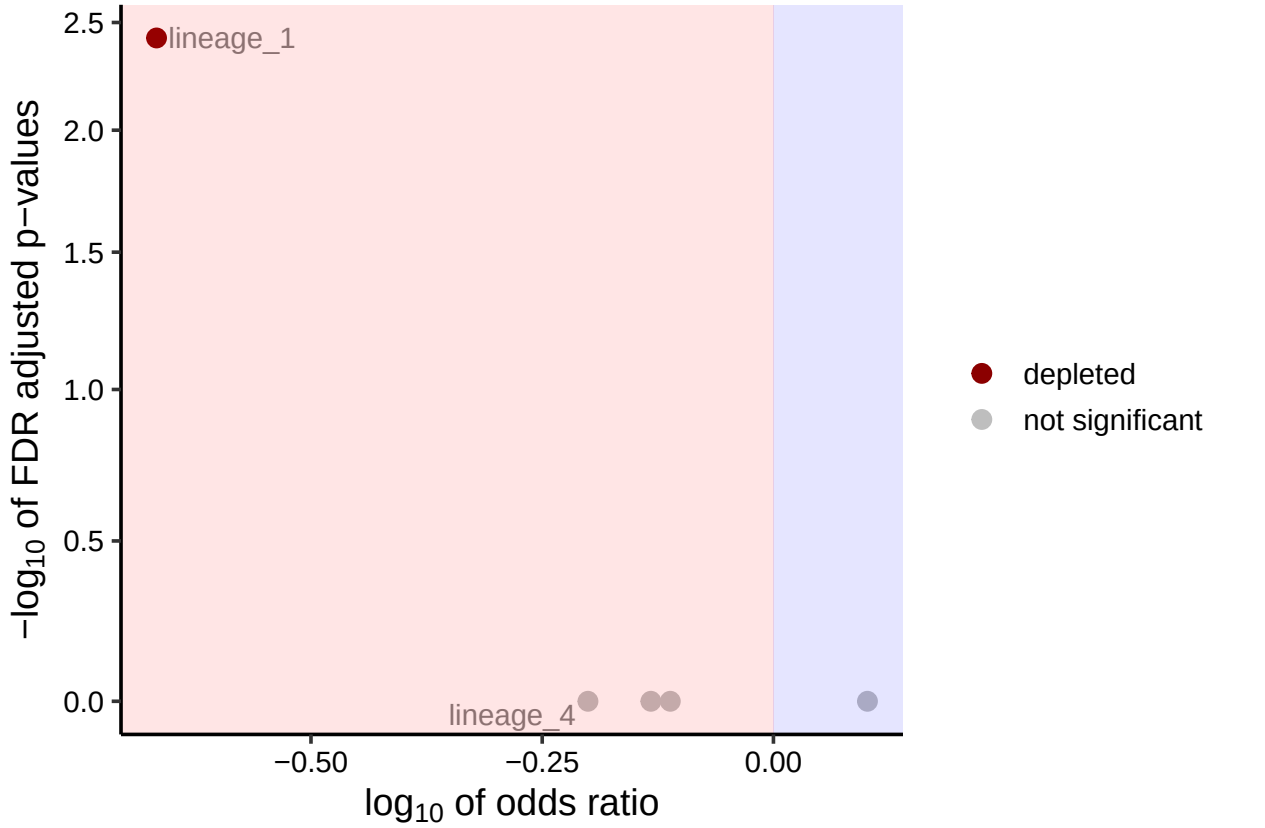
```
##                lineage_1    lineage_2   lineage_3   lineage_4 lineage_5
## cell_151_case 0.00000000 0.0010299588 0.042756294 0.115318029 0.0000000
## cell_152_case 0.83263916 0.0693115699 0.000000000 0.000000000 0.0000000
## cell_153_case 0.07710486 0.0000360329 0.000000000 0.000000000 0.1835377
## cell_154_case 0.65712779 0.0589009524 0.000000000 0.000000000 0.1355032
## cell_155_case 0.13271436 0.0000000000 0.003163341 0.132757013 0.0000000
```

```
## cell_156_case 0.09525577 0.0649880589 0.000000000 0.007613369 0.0000000
##                           id
## cell_151_case cell_151_case
## cell_152_case cell_152_case
## cell_153_case cell_153_case
## cell_154_case cell_154_case
## cell_155_case cell_155_case
## cell_156_case cell_156_case
```

Note that one of the columns is called id and contains the cell names.

We can use the lineage scores and SingleCellExperiments as input to COSICC_DA_lineage.

```
lineage_result <- COSICC_DA_lineage(sce_DA_lineage_case,sce_DA_lineage_control,lineage_scores)
```



The output looks as follows:

```
head(lineage_result)
```

```
##     lineage    p_values odds_ratio            sig
## 1 lineage_1 0.003764327  0.2152840       depleted
## 2 lineage_2 1.000000000  0.7370509 not significant
## 3 lineage_3 1.000000000  1.2637089 not significant
## 4 lineage_4 1.000000000  0.6301841 not significant
## 5 lineage_5 1.000000000  0.7735475 not significant
```