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**celloracle**

***Release 0.5.0***

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CellOracle is a python library for the analysis of Gene Regulatory Network with single cell data.

Source code is available at [celloracle GitHub repository](#)

For more information, please read our bioarxiv preprint: [CellOracle: Dissecting cell identity via network inference and in silico gene perturbation](#)

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**Note:**

Documentation is also available as a pdf file.

[pdf documentation](#)

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**Warning:** CellOracle is still under development. It is beta version and functions in this package may change in the future release.



## CONTENTS

### 1.1 Installation

`celloracle` uses several python libraries and R library. Please follow this guide below to install the dependent software of `celloracle`.

#### 1.1.1 Docker image

- Not available now. Coming soon.

#### 1.1.2 System Requirements

- Operating system: macOS or linux are highly recommended. `celloracle` was developed and tested in Linux and macOS.
- We found that the `celloracle` calculation may be EXTREMELY SLOW under an environment of Windows Subsystem for Linux (WSL). We do not recommend using WSL.
- While you can install `celloracle` in Windows OS, please do so at your own risk and responsibility. We DO NOT provide any support for the use in the Windows OS.
- Memory: 8 G byte or more. Memory usage also depends on your scRNA-seq data. Especially in silico perturbation requires large amount of memory.
- CPU: Core i5 or better processor. GRN inference supports multicore calculation. Higher number of CPU cores enables faster calculation.

#### 1.1.3 Python Requirements

- `celloracle` was developed with python 3.6. We do not support python 2.7x or python <=3.5.
- Please install all dependent libraries before installing `celloracle` according to the instructions below.
- `celloracle` is still beta version and it is not available through PyPI or anaconda distribution yet. Please install `celloracle` from GitHub repository according to the instruction below.

## 0. (Optional) Make a new environment

This step is optional. Please make a new python environment for celloracle and install dependent libraries in it if you get some software conflicts.

```
conda create -n celloracle_env python=3.6
conda activate celloracle_env
```

## 1. Add conda channels

Installation of some libraries requires non-default anaconda channels. Please add the channels below. Instead, you can explicitly enter the channel when you install a library.

```
conda config --add channels defaults
conda config --add channels bioconda
conda config --add channels conda-forge
```

## 2. Install velocyto

Please install velocyto with the following commands or the author's instruction .

```
conda install numpy scipy cython numba matplotlib scikit-learn h5py click pysam llvml
↳louvain
```

Then

```
pip install velocyto
```

It was reported that some compile errors might occur during the installation of velocyto on MacOS. Various errors were reported and you need to find the best solution depending on your error. You may find the solution with these links below.

- Solution 1: Install Xcode. Please try this first.
- Solution 2: Install `macOS_SDK_headers`. This solution is needed in addition to Solution-1 if your OS is MacOS Mojave.
- Solution 3. This is the solution reported by a CellOracle user. Thank you very much!
- Other solutions on [Velocyto github issue page](#)

## 3. Install scanpy

Please install scanpy with the following commands or the author's instruction .

```
conda install scanpy
```

## 4. Install other python libraries

Please install other python libraries below with the following commands.

```
conda install goatools pyarrow tqdm joblib jupyter
```

## 5. install celloracle from github

```
pip install git+https://github.com/morris-lab/CellOracle.git
```

### 1.1.4 R requirements

celloracle use R libraries for the network analysis and scATAC-seq analysis. Please install [R](#) ( $\geq 3.5$ ) and R libraries below according to the author's instruction.

#### Seurat

Please install Seurat with the following r-script or [the author's instruction](#). celloracle is compatible with both Seurat V2 and V3. If you use only scanpy for the scRNA-seq preprocessing and do not use Seurat , you can skip installation of Seurat.

In R console,

```
install.packages('Seurat')
```

#### Cicero

Please install Cicero and Monocle3 with the following r-script or [the author's instruction](#). If you do not have scATAC-seq data and plan to use celloracle's base GRN, you do not need to install Cicero.

In R console,

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install(c("Gviz", "GenomicRanges", "rtracklayer"))

install.packages("devtools")
devtools::install_github("cole-trapnell-lab/cicero-release", ref = "monocle3")
```

#### igraph

Please install igraph with the following r-script or [the author's instruction](#).

In R console,

```
install.packages("igraph")
```

### linkcomm

Please install `linkcomm` with the following r-script or the author's instruction .

In R console,

```
install.packages("linkcomm")
```

### rnetcarto

Please install `rnetcarto` with the following r-script or the author's instruction .

In R console,

```
install.packages("rnetcarto")
```

### Check installation

These R libraries above are necessary for the network analysis in celloracle. You can check installation using celloracle's function.

In python console,

```
import celloracle as co
co.network_analysis.test_R_libraries_installation()
```

Please make sure that all R libraries are installed. The following message will be shown when all R libraries are appropriately installed.

R path: /usr/lib/R/bin/R

```
checking R library installation: igraph -> OK
checking R library installation: linkcomm -> OK
checking R library installation: rnetcarto -> OK
```

The first line above is your R path. If you want to use another R program that was installed at the different place, you can set new R path with the following command.

```
co.network_analysis.set_R_path("ENTER YOUR R PATH HERE")
```

If you changed R path settings, please check installation again to make sure everything works.

```
co.network_analysis.test_R_libraries_installation()
```

## 1.2 Tutorial

The analysis proceeds through multiple steps. Please run the notebooks sequentially. If you do not have ATAC-seq data and want to use the default TF binding information, you can skip the first and second step and start from the third step.

Please refer to the `celloracle` paper for scientific premise and the detail of the algorithm of celloracle.

The jupyter notebook files and data used in this tutorial are available [here](#).

### 1.2.1 ATAC-seq data preprocessing

In this step, we process scATAC-seq data (or bulk ATAC-seq data) to obtain the accessible promoter/enhancer DNA sequence. We can get the active proximal promoter/enhancer genome sequences by picking up the ATAC-seq peaks that exist around the transcription starting site (TSS). Distal cis-regulatory elements can be picked up using [Cicero](#). Cicero analyzes scATAC-seq data to calculate a co-accessible score between peaks. We can identify cis-regulatory elements using Cicero's co-access score and TSS information.

If you have bulk ATAC-seq data instead of scATAC-data, we'll get only the proximal promoter/enhancer genome sequences.

#### A. Extract TF binding information from scATAC-seq data

If you have scATAC-seq data, you can get information on the distal cis-regulatory elements. This step uses Cicero and does not use celloracle. You need to get co-accessibility table in this analysis. Although we provide an example notebook here, you can analyze your data with Cicero in a different way if you are familiar with Cicero. If you have a question about Cicero, please read the documentation of [Cicero](#) for the detailed usage.

#### scATAC-seq analysis with Cicero and Monocle3

The jupyter notebook files and data used in this tutorial are available [here](#).

R notebook

This is an example R script for Cicero analysis. In this R notebook, we'll use Cicero and Monocle3.

Please make sure that you installed these packages in advance.

You can download notebook file and additional data files from celloracle github page. [https://github.com/morris-lab/CellOracle/tree/master/docs/notebooks/01\\_ATAC-seq\\_data\\_processing/option1\\_scATAC-seq\\_data\\_analysis\\_with\\_cicero](https://github.com/morris-lab/CellOracle/tree/master/docs/notebooks/01_ATAC-seq_data_processing/option1_scATAC-seq_data_analysis_with_cicero)

Another tutorial notebook that uses Monocle2 is also available in the celloracle github page above.

#### 0. Import library

```
[2]: library(cicero)
library(monocle3)
```

## 1. Prepare data

In this tutorial we'll use acATAC-seq data from the 10x genomics database. You do not need to download these data if you analyze your own scATAC-seq data.

```
[4]: # Create folder to store data
dir.create("data")

# Download demo dataset from 10x genomics
system("wget -O data/matrix.tar.gz http://cf.10xgenomics.com/samples/cell-atac/1.1.0/
→atac_v1_E18_brain_fresh_5k/atac_v1_E18_brain_fresh_5k_filtered_peak_bc_matrix.tar.gz
→")

# Unzip data
system("tar -xvf data/matrix.tar.gz -C data")
```

```
[6]: # You can substitute the data path below with the data path of your scATAC data.
data_folder <- "data/filtered_peak_bc_matrix"

# Create a folder to save results
output_folder <- "cicero_output"
dir.create(output_folder)
```

## 2. Load data and make Cell Data Set (CDS) object

### 2.1. Process data to make CDS object

```
[7]: # read in matrix data using the Matrix package
indata <- Matrix:::readMM(paste0(data_folder, "/matrix.mtx"))
# binarize the matrix
indata@x[indata@x > 0] <- 1

# format cell info
cellinfo <- read.table(paste0(data_folder, "/barcodes.tsv"))
row.names(cellinfo) <- cellinfo$V1
names(cellinfo) <- "cells"

# format peak info
peakinfo <- read.table(paste0(data_folder, "/peaks.bed"))
names(peakinfo) <- c("chr", "bp1", "bp2")
peakinfo$site_name <- paste(peakinfo$chr, peakinfo$bp1, peakinfo$bp2, sep="_")
row.names(peakinfo) <- peakinfo$site_name

row.names(indata) <- row.names(peakinfo)
colnames(indata) <- row.names(cellinfo)

# make CDS
input_cds <- suppressWarnings(new_cell_data_set(indata,
cell_metadata = cellinfo,
gene_metadata = peakinfo))

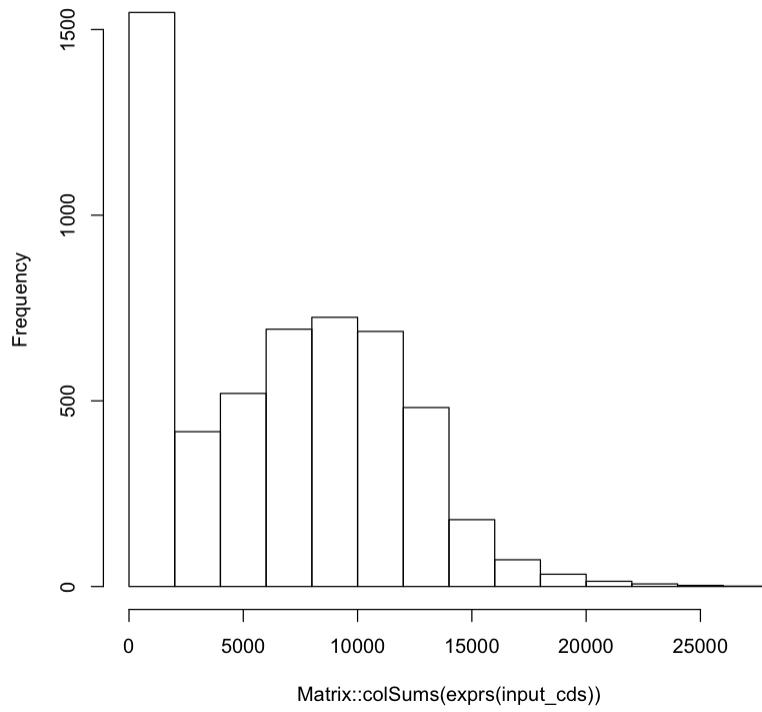
input_cds <- monocle3::detect_genes(input_cds)

#Ensure there are no peaks included with zero reads
input_cds <- input_cds[Matrix:::rowSums(exprs(input_cds)) != 0,]
```

### 3. Quality check and Filtering

```
[8]: # Visualize peak_count_per_cell
hist(Matrix::colSums(exprs(input_cds)))
```

Histogram of Matrix::colSums(exprs(input\_cds))



```
[9]: # filter cells by peak_count
# PLEASE SET APPROPRIATE THRESHOLD VALUES according to your data
max_count <- 15000
min_count <- 2000
input_cds <- input_cds[, Matrix::colSums(exprs(input_cds)) >= min_count]
input_cds <- input_cds[, Matrix::colSums(exprs(input_cds)) <= max_count]
```

### 4. Process cicero-CDS object

```
[ ]: # Data preprocessing
set.seed(2017)

input_cds <- detect_genes(input_cds)
input_cds <- estimate_size_factors(input_cds)
input_cds <- preprocess_cds(input_cds, method = "LSI")

# Dimensional reduction with umap
input_cds <- reduce_dimension(input_cds, reduction_method = 'UMAP',
                                preprocess_method = "LSI")
```

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```
umap_coords <- reducedDims(input_cds)$UMAP

cicero_cds <- make_cicero_cds(input_cds, reduced_coordinates = umap_coords)

# Save cds object if you want
saveRDS(cicero_cds, paste0(output_folder, "/cicero_cds.Rds"))
```

## 5. Load reference genome information

To run cicero, we need to get a genome coordinates files, which contains the lengths of each chromosomes. You can read mm10 genome information with the following command. The text file, mm10\_chromosome\_length.txt is included in the celloracle notebook folder.

[https://github.com/morris-lab/CellOracle/tree/master/docs/notebooks/01\\_ATAC-seq\\_data\\_processing/option1\\_scATAC-seq\\_data\\_analysis\\_with\\_cicero](https://github.com/morris-lab/CellOracle/tree/master/docs/notebooks/01_ATAC-seq_data_processing/option1_scATAC-seq_data_analysis_with_cicero)

If your scATAC-seq data use different reference genome, you need to get a genome coordinates files for your reference genome. Please see the Cicero documentation for more information.

[https://cole-trapnell-lab.github.io/cicero-release/docs\\_m3/#installing-cicero](https://cole-trapnell-lab.github.io/cicero-release/docs_m3/#installing-cicero)

```
[ ]: # !!Please make sure that the reference genome information below match the reference_
  ↪genome of your scATAC-seq data.

# If your scATAC-seq uses mm10 reference genome, you can read chromosome length file_
  ↪with the following command.
chromosome_length <- read.table("./mm10_chromosome_length.txt")

# For mm9 genome, you can use the following command.
#data("mouse.mm9.genome")
#chromosome_length <- mouse.mm9.genome

# For hg19 genome, you can use the following command.
#data("human.hg19.genome")
#chromosome_length <- mhuman.hg19.genome
```

## 6. Run Cicero

```
[11]: # run the main function
conns <- run_cicero(cicero_cds, chromosome_length) # Takes a few minutes to run

# save results
saveRDS(conns, paste0(output_folder, "/cicero_connections.Rds"))

# check results
head(conns)

[1] "Starting Cicero"
[1] "Calculating distance_parameter value"
```

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```
[1] "Running models"
[1] "Assembling connections"
[1] "Done"
```

	Peak1 <fct>	Peak2 <fct>	coaccess <dbl>
A data.frame: 6 × 3	2 chr1_3094484_3095479	chr1_3113499_3113979	-0.316289004
	3 chr1_3094484_3095479	chr1_3119478_3121690	-0.419240532
	4 chr1_3094484_3095479	chr1_3399730_3400368	-0.050867246
	5 chr1_3113499_3113979	chr1_3094484_3095479	-0.316289004
	7 chr1_3113499_3113979	chr1_3119478_3121690	0.370342744
	8 chr1_3113499_3113979	chr1_3399730_3400368	-0.009276026

## 6. Save results for the next step

```
[ ]: all_peaks <- row.names(exprs(input_cds))
write.csv(x = all_peaks, file = paste0(output_folder, "/all_peaks.csv"))
write.csv(x = conns, file = paste0(output_folder, "/cicero_connections.csv"))
```

## TSS annotation

The jupyter notebook files and data used in this tutorial are available [here](#).

Python notebook

In this notebook, we process the results of cicero analysis to get active promoter/enhancer DNA peaks. First, we pick up peaks around the transcription starting site (TSS). Second, we merge cicero data with the peaks around TSS. Then we remove peaks that have a weak connection to TSS peak so that the final product includes TSS peaks and peaks that have a strong connection with the TSS peaks. We use this information as an active promoter/enhancer elements.

## 0. Import libraries

```
[1]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline

import seaborn as sns

import os, sys, shutil, importlib, glob
from tqdm.notebook import tqdm

from celloracle import motif_analysis as ma
```

```
[2]: %config InlineBackend.figure_format = 'retina'

plt.rcParams['figure.figsize'] = [6, 4.5]
plt.rcParams["savefig.dpi"] = 300
```

## 1. Load data made with cicero

```
[3]: # Load all peaks
peaks = pd.read_csv("cicero_output/all_peaks.csv", index_col=0)
peaks = peaks.x.values
peaks

[3]: array(['chr1_3094484_3095479', 'chr1_3113499_3113979',
       'chr1_3119478_3121690', ..., 'chrY_90804622_90805450',
       'chrY_90808626_90809117', 'chrY_90810560_90811167'], dtype=object)

[4]: # Load cicero results
cicero_connections = pd.read_csv("cicero_output/cicero_connections.csv", index_col=0)
cicero_connections.head()

/home/k/anaconda3/envs/test/lib/python3.6/site-packages/numpy/lib/arraysetops.py:568:
  ↪FutureWarning: elementwise comparison failed; returning scalar instead, but in the
  ↪future will perform elementwise comparison
    mask |= (ar1 == a)

[4]:          Peak1            Peak2   coaccess
2  chr1_3094484_3095479  chr1_3113499_3113979 -0.316289
3  chr1_3094484_3095479  chr1_3119478_3121690 -0.419241
4  chr1_3094484_3095479  chr1_3399730_3400368 -0.050867
5  chr1_3113499_3113979  chr1_3094484_3095479 -0.316289
7  chr1_3113499_3113979  chr1_3119478_3121690  0.370343
```

## 2. Make TSS annotation

**IMPORTANT: Please make sure that you are setting correct reference genome.**

If your scATAC-seq data was generated with mm10 reference genome, you can set ref\_genome="mm10". If you used hg19 human reference genome, please set ref\_genome=="hg19"

Currently we support refgenomes below. {"Human": ["hg38", "hg19"], "Mouse": ["mm10", "mm9"], "S.cerevisiae": ["sacCer2", "sacCer3"]}

If your reference genome is not in the list, please send a request through github issue page.

```
[5]: tss_annotated = ma.get_tss_info(peak_str_list=peaks, ref_genome= ) ####! Set reference
  ↪genome here

# Check results
tss_annotated.tail()

que bed peaks: 72402
tss peaks in que: 16987

[5]:      chr      start        end gene_short_name strand
16982  chr1    55130650  55132118        Mob4        +
16983  chr6    94499875  94500767        S1c25a26      +
16984  chr19   45659222  45660823        Fbxw4        -
16985  chr12   100898848 100899597        Gpr68        -
16986  chr4    129491262 129492047        Fam229a      -
```

### 3. Integrate TSS info and cicero connections

The output file after the integration process has three columns; “peak\_id”, “gene\_short\_name”, and “coaccess”. “peak\_id” is either the TSS peak or the peaks that have a connection with the TSS peak. “gene\_short\_name” is the gene name that associated with the TSS site. “coaccess” is the co-access score between a peak and TSS peak. Note, the TSS peak is indicated by a score of 1.

```
[8]: integrated = ma.integrate_tss_peak_with_cicero(tss_peak=tss_annotated,
                                                    cicero_connections=cicero_connections)
print(integrated.shape)
integrated.head()
(263279, 3)

[8]:          peak_id gene_short_name  coaccess
0  chr10_100015291_100017830      Kitl  1.000000
1  chr10_100018677_100020384      Kitl  0.086299
2  chr10_100050858_100051762      Kitl  0.034558
3  chr10_100052829_100053395      Kitl  0.167188
4  chr10_100128086_100128882     Tmtc3  0.022341
```

### 4. Filter peaks

Remove peaks that have weak coaccess score.

```
[9]: peak = integrated[integrated.coaccess >= 0.8]
peak = peak[["peak_id", "gene_short_name"]].reset_index(drop=True)

[10]: print(peak.shape)
peak.head()
(15680, 2)

[10]:          peak_id gene_short_name
0  chr10_100015291_100017830      Kitl
1  chr10_100486534_100488209     Tmtc3
2  chr10_100588641_100589556  4930430F08Rik
3  chr10_100741247_100742505      Gm35722
4  chr10_101681379_101682124     Mgat4c
```

### 5. Save data

Save the promoter/enhancer peak.

```
[11]: peak.to_parquet("peak_file.parquet")
```

-> go to next notebook

## B. Extract TF binding information from bulk ATAC-seq data or Chip-seq data

Bulk DNA-seq data can be used to get the accessible promoter/enhancer sequences.

The jupyter notebook files and data used in this tutorial are available [here](#).

Python notebook

### 0. Import libraries

```
[1]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline

import seaborn as sns

import os, sys, shutil, importlib, glob
from tqdm import tqdm_notebook as tqdm

%config InlineBackend.figure_format = 'retina'

plt.rcParams['figure.figsize'] = [6, 4.5]
plt.rcParams["savefig.dpi"] = 300
```

```
[2]: # Import celloracle function
from celloracle import motif_analysis as ma
```

### 1. Load bed file

Import ATAC-seq bed file. This script can also be used with DNase-seq or Chip-seq data.

```
[3]: file_path_of_bed_file = "data/all_peaks.bed"

[4]: # Load bed_file
bed = ma.read_bed(file_path_of_bed_file)
print(bed.shape)
bed.head()
(436206, 4)

[4]:   chrom      start        end      seqname
0  chr1  3002478  3002968  chr1_3002478_3002968
1  chr1  3084739  3085712  chr1_3084739_3085712
2  chr1  3103576  3104022  chr1_3103576_3104022
3  chr1  3106871  3107210  chr1_3106871_3107210
4  chr1  3108932  3109158  chr1_3108932_3109158
```

```
[6]: # Convert bed file into peak name list
peaks = ma.process_bed_file.df_to_list_peakstr(bed)
peaks

[6]: array(['chr1_3002478_3002968', 'chr1_3084739_3085712',
       'chr1_3103576_3104022', ..., 'chrY_631222_631480',
       'chrY_795887_796426', 'chrY_2397419_2397628'], dtype=object)
```

## 2. Make TSS annotation

IMPORTANT: Please make sure that you are setting the correct ref genome!

```
[7]: tss_annotated = ma.get_tss_info(peak_str_list=peaks, ref_genome="mm9")

# Check results
tss_annotated.tail()

que bed peaks: 436206
tss peaks in que: 24822

[7]:
```

	chr	start	end	gene_short_name	strand
24817	chr2	60560211	60561602	Itgb6	-
24818	chr15	3975177	3978654	BC037032	-
24819	chr14	67690701	67692101	Ppp2r2a	-
24820	chr17	48455247	48455773	B430306N03Rik	+
24821	chr10	59861192	59861608	Gm17455	+

```
[9]: # Change format
peak_id_tss = ma.process_bed_file.df_to_list_peakstr(tss_annotated)
tss_annotated = pd.DataFrame({ "peak_id": peak_id_tss,
                               "gene_short_name": tss_annotated.gene_short_name.values}
                             )
tss_annotated = tss_annotated.reset_index(drop=True)
print(tss_annotated.shape)
tss_annotated.head()

(24822, 2)

[9]:
```

	peak_id	gene_short_name
0	chr7_50691730_50692032	Nkg7
1	chr7_50692077_50692785	Nkg7
2	chr13_93564413_93564836	Thbs4
3	chr13_14613429_14615645	Hecw1
4	chr3_99688753_99689665	Spag17

## 3. Save data

```
[10]: tss_annotated.to_parquet("peak_file.parquet")
```

-> go to next notebook

### 1.2.2 Transcription factor binding motif scan

We identified accessible Promoter/enhancer DNA regions using ATAC-seq data. Next, we will obtain a list of TFs for each target gene by scanning the regulatory genomic sequences for TF-binding motifs. In the later GRN inference process, this list will be used to define potential regulatory connections.

The jupyter notebook files and data used in this tutorial are available [here](#).

## Scan DNA sequences searching for TF binding motifs

Python notebook

### 0. Import libraries

```
[1]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt

import seaborn as sns

import os, sys, shutil, importlib, glob
from tqdm.notebook import tqdm
```

```
[2]: from celloracle import motif_analysis as ma
from celloracle.utility import save_as_pickled_object
```

```
[3]: %config InlineBackend.figure_format = 'retina'
%matplotlib inline

plt.rcParams['figure.figsize'] = (15, 7)
plt.rcParams["savefig.dpi"] = 600
```

## 1. Reference genome data preparation

### 1.1. Check reference genome installation

Celloracle uses genomepy to get DNA sequence data. Before starting celloracle analysis, we need to make sure that the reference genome is correctly installed in your computational environment. If not, please install reference genome.

```
[4]: # PLEASE make sure that you are setting correct ref genome.
ref_genome = "mm10"

genome_installation = ma.is_genome_installed(ref_genome=ref_genome)
print(ref_genome, "installation:", genome_installation)

mm10 installation: True
```

### 1.2. Install reference genome (if refgenome is not installed)

```
[5]: if not genome_installation:
    import genomepy
    genomepy.install_genome(ref_genome, "UCSC")
else:
    print(ref_genome, "is installed.")

mm10 is installed.
```

## 2. Load data

### 2.1. Load processed peak data

```
[6]: # Load annotated peak data.
peaks = pd.read_parquet("../01_ATAC-seq_data_processing/option1_scATAC-seq_data_"
                       "analysis_with_cicero/peak_file.parquet")
peaks.head()

[6]:
          peak_id gene_short_name
0  chr10_100015291_100017830        Kitl
1  chr10_100486534_100488209        Tmtc3
2  chr10_100588641_100589556  4930430F08Rik
3  chr10_100741247_100742505        Gm35722
4  chr10_101681379_101682124        Mgat4c
```

### 2.1. Check data

```
[7]: # Define function for quality check
def decompose_chrstr(peak_str):
    """
    Args:
        peak_str (str): peak_str. e.g. 'chr1_3094484_3095479'

    Returns:
        tuple: chromosome name, start position, end position
    """
    *chr_, start, end = peak_str.split("_")
    chr_ = "_".join(chr_)
    return chr_, start, end

from genomepy import Genome

def check_peak_fomat(peaks_df, ref_genome):
    """
    Check peak fomat.
    (1) Check chromosome name.
    (2) Check peak size (length) and remove sort DNAs (<5bp)

    """
    df = peaks_df.copy()

    n_peaks_before = df.shape[0]

    # Decompose peaks and make df
    decomposed = [decompose_chrstr(peak_str) for peak_str in df["peak_id"]]
    df_decomposed = pd.DataFrame(np.array(decomposed))
    df_decomposed.columns = ["chr", "start", "end"]
    df_decomposed["start"] = df_decomposed["start"].astype(np.int)
    df_decomposed["end"] = df_decomposed["end"].astype(np.int)

    # Load genome data
    genome_data = Genome(ref_genome)
```

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```

all_chr_list = list(genome_data.keys())

# DNA length check
lengths = np.abs(df_decomposed["end"] - df_decomposed["start"])

# Filter peaks with invalid chromosome name
n_threshold = 5
df = df[(lengths >= n_threshold) & df_decomposed.chr.isin(all_chr_list)]

# DNA length check
lengths = np.abs(df_decomposed["end"] - df_decomposed["start"])

# Data counting
n_invalid_length = len(lengths[lengths < n_threshold])
n_peaks_invalid_chr = n_peaks_before - df_decomposed.chr.isin(all_chr_list).sum()
n_peaks_after = df.shape[0]

#
print("Peaks before filtering: ", n_peaks_before)
print("Peaks with invalid chr_name: ", n_peaks_invalid_chr)
print("Peaks with invalid length: ", n_invalid_length)
print("Peaks after filtering: ", n_peaks_after)

return df

```

```
[8]: peaks = check_peak_fomat(peaks, ref_genome)

Peaks before filtering: 15900
Peaks with invalid chr_name: 0
Peaks with invalid length: 2
Peaks after filtering: 15898
```

## 2.2. [Optional step] Load motifs

You can select TF binding motif data for Celloracle motif analysis. If you have no preference and just want to use a default motif, you can skip this step. If you want to use a non-default motif dataset, please prepare motif data as a list of motif class in gimmermotifs. We have several option for loading motif database as below.

### 2.2.1. [Optional step] Load motif data from gimmermotifs dataset

Many motif databases are included with GimmeMotifs. <https://gimmermotifs.readthedocs.io/en/master/overview.html>  
You can load them as follows.

```
[9]: # First, we need to pick up motifs for your dataset. We can get a list.

# Get folder path that stores motif data.
import os, glob
from gimmermotifs.motif import MotifConfig
config = MotifConfig()
motif_dir = config.get_motif_dir()
```

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```
# Get list for motif data name
motifs_data_name = [i for i in os.listdir(motif_dir) if i.endswith(".pfm")]
motifs_data_name.sort()
motifs_data_name
```

[9]:

```
['CIS-BP.pfm',
 'ENCODE.pfm',
 'HOCOMOCov10_HUMAN.pfm',
 'HOCOMOCov10_MOUSE.pfm',
 'HOCOMOCov11_HUMAN.pfm',
 'HOCOMOCov11_MOUSE.pfm',
 'HOMER.pfm',
 'IMAGE.pfm',
 'JASPAR2018.pfm',
 'JASPAR2018_fungi.pfm',
 'JASPAR2018_insects.pfm',
 'JASPAR2018_nematodes.pfm',
 'JASPAR2018_plants.pfm',
 'JASPAR2018_urochordates.pfm',
 'JASPAR2018_vertebrates.pfm',
 'JASPAR2020.pfm',
 'JASPAR2020_fungi.pfm',
 'JASPAR2020_insects.pfm',
 'JASPAR2020_nematodes.pfm',
 'JASPAR2020_plants.pfm',
 'JASPAR2020_urochordates.pfm',
 'JASPAR2020_vertebrates.pfm',
 'RSAT_insects.pfm',
 'RSAT_plants.pfm',
 'RSAT_vertebrates.pfm',
 'SwissRegulon.pfm',
 'factorbook.pfm',
 'gimme.vertebrate.v5.0.pfm']
```

[10]:

```
# Once you picked up motifs, you can load the motif files with "read_motifs"
from gimmermotifs.motif import read_motifs

path = os.path.join(motif_dir, "JASPAR2018_plants.pfm") # Please enter motifs name here
motifs = read_motifs(path)

# Check first 10 motifs
motifs[:10]
```

[10]:

```
[MA0020.1_Dof2_AAAGCn,
 MA0021.1_Dof3_AAAGyn,
 MA0034.1_Gam1_nnyAACCGmC,
 MA0044.1_HMG-1_sTTGTnyTy,
 MA0045.1_HMG-I/Y_nwAnAAAnrnmrAmAy,
 MA0053.1_MNB1A_AAAGC,
 MA0054.1_myb.Ph3_TAACnGTTw,
 MA0064.1_PBF_AAAGY,
 MA0082.1_squamosa_mC AwAwATrGwAAn,
 MA0096.1_bZIP910_mTGACGT]
```

## 2.2.2 [Optional step] Load motif data from celloracle motif dataset

Celloracle also provides many motif dataset that was generated from CisDB. <http://cisbp.ccbr.utoronto.ca/>

These motifs were organized by each species. Please select motifs for your species.

If you have a request for motifs for a new species, you can ask us to add new motifs through github issue page.

```
[11]: # Check available motifs
ma.MOTIFS_LIST

[11]: ['CisDB_ver2_Mus_musculus.pfm',
       'CisDB_ver2_Saccharomyces_cerevisiae.pfm',
       'CisDB_ver2_Danio_rerio.pfm',
       'CisDB_ver2_Homo_sapiens.pfm']

[12]: # Load motifs from celloracle dataset.
motifs = ma.load_motifs("CisDB_ver2_Mus_musculus.pfm")

# Check first 10 motifs
motifs[:10]

[12]: [M00008_2.00_nnnAAww,
      M00044_2.00_nrTAAACAn,
      M00056_2.00_TAATAAAT,
      M00060_2.00_nnnTTCnnn,
      M00111_2.00_nGCCynnGGs,
      M00112_2.00_CCTsrGGCnA,
      M00113_2.00_nsCCnnAGGs,
      M00114_2.00_nnGCCynnGG,
      M00115_2.00_nnATnAAAn,
      M00116_2.00_nnAATATTAnn]
```

## 3. Instantiate TFinfo object and search for TF binding motifs

The motif analysis module has a custom class; TFinfo. The TFinfo object converts a peak data into a DNA sequences and scans the DNA sequences searching for TF binding motifs. Then, the results of motif scan will be filtered and converted into either a python dictionary or a depending on your preference. This TF information is necessary for GRN inference.

### 3.1. Instantiate TFinfo object

```
[16]: # Instantiate TFinfo object
tfi = ma.TFinfo(peak_data_frame=peaks, # peak info calculated from ATAC-seq data
                 ref_genome=ref_genome)
```

### 3.2. Motif scan

!!You can set TF binding motif information as an argument:

```
tfi.scan(motifs=motifs)
```

If you don't set motifs or set None, default motifs will be loaded automatically.

- For mouse and human, “gimme.vertebrate.v5.0.” will be used as a default motifs.
- For another species, a species specific TF binding motif data extracted from CisDB ver2.0 will be used.

```
[ ]: %%time
# Scan motifs. !!CAUTION!! This step may take several hours if you have many peaks!
tfi.scan(fpr=0.02,
         motifs=motifs, # If you enter None, default motifs will be loaded.
         verbose=True)

# Save tfinfo object
tfi.to_hdf5(file_path="test1.celloracle.tfinfo")
```

```
[16]: # Check motif scan results
tfi.scanned_df.head()
```

	seqname	motif_id	factors_direct	\
0	chr10_100015291_100017830	GM.5.0.Homeodomain.0001	TGIF1	
1	chr10_100015291_100017830	GM.5.0.Mixed.0001		
2	chr10_100015291_100017830	GM.5.0.Mixed.0001		
3	chr10_100015291_100017830	GM.5.0.Mixed.0001		
4	chr10_100015291_100017830	GM.5.0.Nuclear_receptor.0002	NR2C2	

	factors_indirect	score	pos	strand
0	ENSG00000234254, TGIF1	10.311002	1003	1
1	SRF, EGR1	7.925873	481	1
2	SRF, EGR1	7.321375	911	-1
3	SRF, EGR1	7.276585	811	-1
4	NR2C2, Nr2c2	9.067331	449	-1

We have the score for each sequence and motif\_id pair. In the next step we will filter the motifs with low score.

### 5. Filtering motifs

```
[15]: # Reset filtering
tfi.reset_filtering()

# Do filtering
tfi.filter_motifs_by_score(threshold=10.5)

# Do post filtering process. Convert results into several file format.
```

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```
tfi.make_TFinfo_dataframe_and_dictionary(verbose=True)

peaks were filtered: 12952283 -> 2288874
1. converting scanned results into one-hot encoded dataframe.

HBox(children=(FloatProgress(value=0.0, max=14142.0), HTML(value='')))

2. converting results into dictionaries.
converting scan results into dictionaries...

HBox(children=(FloatProgress(value=0.0, max=15006.0), HTML(value='')))

HBox(children=(FloatProgress(value=0.0, max=1090.0), HTML(value='')))
```

## 6. Get Final results

### 6.1. Get results as a dictionary

```
[23]: td = tfi.to_dictionary(dictionary_type="targetgene2TFs")
```

### 6.2. Get results as a dataframe

```
[17]: df = tfi.to_dataframe()
df.head()

[17]:      peak_id gene_short_name  9430076c15rik  Ac002126.6 \
0  chr10_100015291_100017830          Kitl        0.0        0.0
1  chr10_100486534_100488209         Tmtc3        0.0        0.0
2  chr10_100588641_100589556  4930430F08Rik        0.0        0.0
3  chr10_100741247_100742505         Gm35722        0.0        0.0
4  chr10_101681379_101682124        Mgat4c        0.0        0.0

   Ac012531.1  Ac226150.2  Afp  Ahr  Ahrr  Aire  ...  Znf784  Znf8  Znf816 \
0        0.0        0.0  0.0  1.0  1.0  0.0  ...    0.0  0.0        0.0
1        0.0        0.0  0.0  0.0  0.0  0.0  ...    1.0  0.0        0.0
2        1.0        0.0  0.0  1.0  1.0  0.0  ...    0.0  0.0        0.0
3        0.0        0.0  0.0  0.0  0.0  0.0  ...    0.0  0.0        0.0
4        0.0        0.0  0.0  0.0  0.0  0.0  ...    0.0  0.0        0.0

  Znf85  Zscan10  Zscan16  Zscan22  Zscan26  Zscan31  Zscan4
0    0.0    0.0    0.0    0.0    0.0    1.0    0.0
1    0.0    0.0    0.0    1.0    0.0    0.0    0.0
2    0.0    0.0    0.0    0.0    0.0    0.0    0.0
3    0.0    0.0    0.0    0.0    0.0    0.0    0.0
4    0.0    0.0    0.0    0.0    0.0    0.0    1.0

[5 rows x 1092 columns]
```

## 7. Save TFinfo as dictionary or dataframe

We'll use this information when making the GRNs. Save the results.

```
[19]: folder = "TFinfo_outputs"
os.makedirs(folder, exist_ok=True)

# save TFinfo as a dictionary
td = tfi.to_dictionary(dictionary_type="targetgene2TFs")
save_as_pickled_object(td, os.path.join(folder, "TFinfo_targetgene2TFs.pickled"))

# save TFinfo as a dataframe
df = tfi.to_dataframe()
df.to_parquet(os.path.join(folder, "TFinfo_dataframe.parquet"))
```

[ ]:

### [Optional step 1] How to use different motif data

Celloracle motif analysis pipeline provides several default motifs. If you don't enter motif data, celloracle automatically load default motifs for your species. In that case, you don't need to make TF binding motifs. But also, you can pick up TF binding motifs by yourself. Here, we introduce how to find and load motifs for celloracle motif analysis. Some codes for custom motif were based on the suggestion in [this post](#). from KyleFerchen. Thank you Kyle!

## 0. Overview: How to use a different motifs for celloracle motif scan.

In this notebook, we introduce how to prepare motif dataset for celloracle motif analysis. Celloracle uses list of motif object in `gimmemotifs` package. See `gimmemotifs` documentation for more details. (<https://gimmemotifs.readthedocs.io/en/master/api.html#>)

### 1 Import motifs from `gimmemotifs` dataset.

`Gimmemotifs` provides many motif dataset that was generated from public motif database including CisDB, ENCODE, HOMER, and JASPAR. <https://gimmemotifs.readthedocs.io/en/master/overview.html>

#### 1.1 `gimme.vertebrate.v5.0`.

By default GimmeMotifs uses a non-redundant, clustered database of known vertebrate motifs. These motifs come from CIS-BP (<http://cisbp.ccbr.utoronto.ca/>) and other sources. This motif dataset can be easily loaded with the following command.

If your dataset is Mouse or Human, this one will be a good default choice.

```
[1]: # Compare with default motifs in gimmemotifs
from gimmemotifs.motif import default_motifs
motifs = default_motifs()

# Check first 10 motifs
motifs[:10]
```

```
/home/k/anaconda3/envs/pandas1/lib/python3.6/site-packages/gimmemotifs/plot.py:19:  
  ↪MatplotlibDeprecationWarning: The 'warn' parameter of use() is deprecated since  
  ↪Matplotlib 3.1 and will be removed in 3.3. If any parameter follows 'warn', they  
  ↪should be pass as keyword, not positionally.  
    mpl.use("Agg", warn=False)
```

```
[1]: [GM.5.0.Sox.0001_AACAAT,  
 GM.5.0.Homeodomain.0001_AGCTGTCAnnA,  
 GM.5.0.Mixed.0001_snnGGssssGGs,  
 GM.5.0.Nuclear_receptor.0001_TAwsTrGGTCAsTrGGTCA,  
 GM.5.0.Mixed.0002_GCTAATTA,  
 GM.5.0.Nuclear_receptor.0002_wnyrrCTTCCGGGkC,  
 GM.5.0.bHLH.0001_ACGTG,  
 GM.5.0.Myb_SANT.0001_rrCCGTTAACnGyy,  
 GM.5.0.C2H2_ZF.0001_GCGkGGGCAG,  
 GM.5.0.GATA.0001_TTATCTSnnnnnnnnCA]
```

## 1.2 Motifs that are provided with gimmemotifs package

Many other motif databases come included with GimmeMotifs. You can load them as follows.

```
[26]: # Get folder path that stores motif data.  
import os, glob  
from gimmemotifs.motif import MotifConfig  
config = MotifConfig()  
motif_dir = config.get_motif_dir()  
  
# Get motif data names  
motifs_data_name = [i for i in os.listdir(motif_dir) if i.endswith(".pfm")]  
motifs_data_name.sort()  
motifs_data_name  
  
[26]: ['CIS-BP.pfm',  
       'ENCODE.pfm',  
       'HOCOMOCOV10_HUMAN.pfm',  
       'HOCOMOCOV10_MOUSE.pfm',  
       'HOCOMOCOV11_HUMAN.pfm',  
       'HOCOMOCOV11_MOUSE.pfm',  
       'HOMER.pfm',  
       'IMAGE.pfm',  
       'JASPAR2018.pfm',  
       'JASPAR2018_fungi.pfm',  
       'JASPAR2018_insects.pfm',  
       'JASPAR2018_nematodes.pfm',  
       'JASPAR2018_plants.pfm',  
       'JASPAR2018_urochordates.pfm',  
       'JASPAR2018_vertebrates.pfm',  
       'RSAT_insects.pfm',  
       'RSAT_plants.pfm',  
       'RSAT_vertebrates.pfm',  
       'SwissRegulon.pfm',  
       'factorbook.pfm',  
       'gimme.vertebrate.v5.0.pfm']
```

```
[30]: # You can load motif files with "read_motifs"  
from gimmemotifs.motif import read_motifs
```

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```
path = os.path.join(motif_dir, "JASPAR2018_plants.pfm")
motifs = read_motifs(path)

# Check first 10 motifs
motifs[:10]

[30]: [MA0020_1_Dof2_AAAGCn,
MA0021_1_Dof3_AAAGyn,
MA0034_1_Gam1_nnyAACCGmC,
MA0044_1_HMG-1_sTTGTnyTy,
MA0045_1_HMG-I/Y_nwAnAAAnrnmrAmAy,
MA0053_1_MNB1A_AAAGC,
MA0054_1_myb.Ph3_TAACnGTTw,
MA0064_1_PBF_AAAGY,
MA0082_1_squamosa_mC AwAwATrGwAAn,
MA0096_1_bZIP910_mTGACGT]
```

## 2. Import motifs from Celloracle dataset.

Celloracle provides many motif dataset that was generated from CisDB. These motifs were divided by species. Please select motifs for your species.

```
[37]: from celloracle import motif_analysis as ma

# Check available motifs
ma.MOTIFS_LIST

[37]: ['CisDB_ver2_Mus_musculus.pfm',
'CisDB_ver2_Saccharomyces_cerevisiae.pfm',
'CisDB_ver2_Danio rerio.pfm',
'CisDB_ver2_Homo_sapiens.pfm']

[39]: # Load motifs from celloracle dataset.
motifs = ma.load_motifs("CisDB_ver2_Homo_sapiens.pfm")

# Check first 10 motifs
motifs[:10]

[39]: [M00056_2.00_TAATAAAT,
M00070_2.00_nrAACAAATAnn,
M00111_2.00_nGCCynnGGs,
M00112_2.00_CCTsrGGCnA,
M00113_2.00_nsCCnnAGGs,
M00114_2.00_nnGCCynnGG,
M00115_2.00_nnATnAAAn,
M00116_2.00_nnAATATTAnn,
M00130_2.00_nnnGCCnCnn,
M00142_2.00_GTrCTCmy]
```

### 3. Import motifs from custom motif dataset.

If you want to use another motif data source, you need to make a list of motif class in `gimmemotifs`. The easiest way to make such object is to use “`read_motifs`” function in `gimmemotifs`.

This function can load motif data text file. You need to prepare two files, `XXX.motif2factors.txt` and `XXX.pfm`.

#### 3.1 XXX.motif2factors.txt

The text file, `XXX.motif2factors.txt` includes TF factor annotation for each motifs. The file should be like a tsv file like below.

The first column should be motif name, the motif name should match with motif name in pfm file. The second column is gene symbol, the third column is datasource. This column is not important. The forth column is additional information for this factor. The factor is labeled with “Y” If factor information was confirmed by some evidence. Otherwise, the factor is labeled with “N”.

```
[52]: path_motif2factors = path.replace(".pfm", ".motif2factors.txt")
```

```
with open(path_motif2factors, "r") as f:
    for i, j in enumerate(f):
        print(j)
        if i>5:
            break
```

Motif	Factor	Evidence	Curated
MA0020.1_Dof2	Dof2	SELEX	Y
MA0021.1_Dof3	Dof3	SELEX	Y
MA0034.1_Gam1	Gam1	SELEX	Y
MA0044.1_HMG-1	HMG-1	SELEX	Y
MA0045.1_HMG-I/Y		HMG-I/Y	SELEX Y
MA0053.1_MNB1A	MNB1A	SELEX	Y

#### 3.2 XXX.pfm

The second file, `XXX.pfm`. should includes motif pwm information. The file shoud be like below.

The motif name in this pfm file should exactly match with the motif name in `motif2factor.txt` file.

```
[55]: with open(path, "r") as f:
    for i, j in enumerate(f):
        print(j)
        if i>10:
            break
```

```
# JASPAR2018_plants motif database
```

```
# Retrieved from: http://jaspar.genereg.net/download/CORE/JASPAR2018_CORE_plants_non-
˓→redundant_pfms_jaspar.txt
```

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```
# Date: 2018-10-17

>MA0020.1_Dof2

0.9999  0.0000  0.0000  0.0000
0.9999  0.0000  0.0000  0.0000
0.9999  0.0000  0.0000  0.0000
0.0000  0.0000  0.9999  0.0000
0.1429  0.6666  0.0953  0.0953
0.3333  0.2857  0.1429  0.2381

>MA0021.1_Dof3

0.9999  0.0000  0.0000  0.0000
```

### 3.3 Load files as motif list

We can load files using `read_motifs` function in `gimmemotifs`.

First, please prepare two files, `XXX.motif2factors.txt` and `XXX.pfm`. in the same directly. If you have theses two file in a different place, we cannot use the `read_motifs` function.

Then use file path for `XXX.pfm` for the argument of `read_motifs` function.

```
[58]: from gimmemotifs.motif import read_motifs

# Check path for pfm file
print(path)

# Read motifs
motifs = read_motifs(path)

# Check first 10 motifs
motifs[:10]
/home/k/anaconda3/envs/pandas1/lib/python3.6/site-packages/data/motif_databases/
↳JASPAR2018_plants.pfm
```

```
[58]: [MA0020.1_Dof2_AAAGCn,
MA0021.1_Dof3_AAAGyn,
MA0034.1_Gam1_nnyAACCGmC,
MA0044.1_HMG-1_sTTGTnyTy,
MA0045.1_HMG-I/Y_nwAnAAAnrnmrAmAy,
MA0053.1_MNB1A_AAAGC,
MA0054.1_myb.Ph3_TAACnGTTw,
MA0064.1_PBF_AAAGY,
MA0082.1_squamosa_mC AwAwATrGwAAn,
MA0096.1_bZIP910_mTGACGT]
```

In another notebook, we introduce how to make XXX.pfm file and XXX.motif2factors.txt file. Please look at that if you want to make your motif data by yourself.

### [Optional step 2] How to Make custom motifs for celloracle motif analysis

If you cannot find an appropriate motif dataset for your analysis and want to do it by yourself. You can follow the instruction below. We introduce an example way to make motifs using [CisDB TF binding database](#).

#### 0. Overview: How to Make custom motifs for celloracle motif scan.

In this notebook, we introduce how to make motif dataset for celloracle motif analysis. Celloracle uses list of motif object in `gimmemotifs` package. See `gimmemotifs` documentation for more details. (<https://gimmemotifs.readthedocs.io/en/master/api.html#>)

Here, we get motif data from CisBP (version2).<http://cisbp.ccbr.utoronto.ca>

We will extract motif information for a specific species and save as XXX.pfm and XXX.motif2factors.txt file. These files can be read with `read_motifs` function in `gimmemotifs`.

[1]:

```
import numpy as np
import pandas as pd
import seaborn as sns
import sys, os

import matplotlib.pyplot as plt
%matplotlib inline
plt.rcParams["figure.figsize"] = [20,10]

from gimmemotifs.motif import Motif, read_motifs
```

[ ]:

#### 1. Download full dataset for TF dataset from CisBP database

Go the URL below for the download link for the latest data. <http://cisbp.ccbr.utoronto.ca/entireDownload.php>

[ ]:

```
! wget http://cisbp.ccbr.utoronto.ca/data/2.00/DataFiles/Bulk_downloads/EntireDataset/
→PWMs.zip
! unzip PWMs.zip
```

[3]:

```
!wget http://cisbp.ccbr.utoronto.ca/data/2.00/DataFiles/Bulk_downloads/EntireDataset/
→TF_Information_all_motifs.txt.zip
! unzip TF_Information_all_motifs.txt.zip

--2020-08-03 16:34:06-- http://cisbp.ccbr.utoronto.ca/data/2.00/DataFiles/Bulk_
→downloads/EntireDataset/TF_Information_all_motifs.txt.zip
Resolving cisbp.ccbr.utoronto.ca (cisbp.ccbr.utoronto.ca)... 142.150.52.218
Connecting to cisbp.ccbr.utoronto.ca (cisbp.ccbr.utoronto.ca)|142.150.52.218|:80...
→connected.
HTTP request sent, awaiting response... 200 OK
Length: 115455298 (110M) [application/zip]
Saving to: 'TF_Information_all_motifs.txt.zip'
```

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```
TF_Information_all_ 100%[=====] 110.11M 11.1MB/s    in 19s
2020-08-03 16:34:25 (5.73 MB/s) - 'TF_Information_all_motifs.txt.zip' saved
↪ [115455298/115455298]
Archive: TF_Information_all_motifs.txt.zip
inflating: TF_Information_all_motifs.txt
```

```
[4]: # Load TF information as a dataframe.
df = pd.read_table("TF_Information_all_motifs.txt")
df.head()

/home/k/anaconda3/envs/pandas1/lib/python3.6/site-packages/IPython/core/
↪ interactiveshell.py:3072: DtypeWarning: Columns (19) have mixed types.Specify dtype_
↪ option on import or set low_memory=False.
    interactivity=interactivity, compiler=compiler, result=result)

[4]:
```

	TF_ID	Family_ID	TSource_ID	Motif_ID	MSource_ID	DBID	\
0	T000001_2.00	F001_2.00	TS12_2.00	.	.	BRADI2G60554	
1	T000002_2.00	F001_2.00	TS12_2.00	.	.	LPERR05G06870	
2	T000003_2.00	F002_2.00	TS04_2.00	.	.	CPAG_02544	
3	T000004_2.00	F002_2.00	TS04_2.00	.	.	PTSG_00627	
4	T000005_2.00	F002_2.00	TS04_2.00	.	.	WUBG_06707	

	TF_Name	TF_Species	TF_Status	Family_Name	...	\
0	BRADI2G60554	Brachypodium_distachyon	N	ABF1,B3	...	
1	LPERR05G06870	Leersia_perrieri	N	ABF1,B3	...	
2	CPAG_02544	Candida_parapsilosis	N	ABF1	...	
3	PTSG_00627	Salpingoeca_rosetta	N	ABF1	...	
4	WUBG_06707	Wuchereria_bancrofti	N	ABF1	...	

	MSource_Year	PMID	MSource_Version	SR_Model	SR_NoThreshold	\
0	.	.	.	SequenceIdentity	True	
1	.	.	.	SequenceIdentity	True	
2	.	.	.	SequenceIdentity	True	
3	.	.	.	SequenceIdentity	True	
4	.	.	.	SequenceIdentity	True	

	TfSource_Name	TfSource_URL	TfSource_Year	TfSource_Month	\
0	Ensembl	http://www.ensembl.org/	2018	Dec	
1	Ensembl	http://www.ensembl.org/	2018	Dec	
2	Broad	http://www.broadinstitute.org/	2016	May	
3	Broad	http://www.broadinstitute.org/	2016	May	
4	Broad	http://www.broadinstitute.org/	2016	May	

	TfSource_Day
0	8
1	8
2	1
3	1
4	1

[5 rows x 28 columns]

```
[14]: df.shape
```

[14]: (10879322, 28)

## 2. Define custom functions

```
[15]: # All process will be done inside these function.

from datetime import datetime
import glob

def read_pwm_and_convert_into_list(path):
    # read pwm as df
    pwm = pd.read_csv(path, delimiter="\t")

    # convert into list of str
    li = []
    for i in pwm.iterrows():
        i = i[1].values[1:]
        i = "\t".join(i.astype("str")) + "\n"
        li.append(i)

    return li

def make_motif_file_from_cisbp_data(pwm_folder_path, tfinfo_df, species):

    data_ = tfinfo_df[tfinfo_df.TF_Species == species]
    data_name = "CisDB_ver2_" + species

    ## 1. Make file: motif2factors.txt

    # Select information
    df_factors = data_[["Motif_ID", "TF_Name", "MSource_Type", "TF_Status"]]
    df_factors = df_factors[df_factors.TF_Status != "N"]

    # Formatting
    df_factors.columns = 'Motif\tFactor\tEvidence\tCurated'.split("\t")
    df_factors["Curated"] = [{"D": "Y", "I": "N"}[i] for i in df_factors["Curated"]]
    df_factors = df_factors.sort_values(by="Motif")

    ## 2. Make file: pfm file
    comments = f"# CIS-BP motif database (v2.0), retrieved by Celloracle\n"
    comments += "# Retrieved from: http://cisbp.ccb.utoronto.ca/data/2.00/DataFiles/\n"
    comments += "Bulk_downloads/EntireDataset/PWMs.zip\n"
    comments += f"#Date: {datetime.now().ctime()}\n"

    # Get list of motif name
    paths_pwm = glob.glob(os.path.join(pwm_folder_path, "*.txt"))
    paths_pwm.sort()
    motif_names = [path.split("/")[-1].replace(".txt", "") for path in paths_pwm]
    motifs = np.intersect1d(motif_names, df_factors.Motif.unique())

    print(motifs.shape)

    # Intersect motif information with pwm information
    df_factors = df_factors[df_factors.Motif.isin(motifs)]
```

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```

# Load, convert, and save pwm info
output = data_name + ".pfm"

motifs_non_zero = []
with open(output, "w") as f:

    for motif_name in motifs:

        path = os.path.join(pwm_folder_path, motif_name + ".txt")
        pwm = read_pwn_and_convert_into_list(path=path) # Load and convert
        if pwm:
            motifs_non_zero.append(motif_name)
            pwm = [f">{motif_name}\n"] + pwm
            for i in pwm: # Save pfm
                f.write(i)

# Intersect motif information with pwm information
df_factors = df_factors[df_factors.Motif.isin(motifs_non_zero)] 

# Save factor info
df_factors.to_csv(f'{data_name}.motif2factors.txt', sep='\t', index=False)

print(df_factors.shape, len(motifs_non_zero))

```

### 3. Pick up motif information for one species and save as gmmemotif pfm file format.

```
[17]: # Check species in this dataset
species_list = df.TF_Species.unique()
species_list.sort()
```

```

for i in species_list:
    print(i)

Acanthamoeba_castellani
Acanthamoeba_polyphaga_mimivirus
Acanthocheilonema_viteae
Acipenser_baerii
Acremonium_chrysogenum
Acropora_formosa
Acropora_millepora
Acyrthosiphon_pisum
Aedes_aegypti
Aegilops_tauschii
Agaricus_bisporus
Ailuropoda_melanoleuca
Albugo_laibachii
Alligator_sinensis
Allomyces_macrogyrus
Alternaria_brassicicola
Amanita_muscaria
Amborella_trichopoda
Amphimedon_queenslandica
Anas_platyrhynchos

```

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Ancylostoma\_caninum  
Ancylostoma\_ceylanicum  
Ancylostoma\_duodenale  
Angiostrongylus\_cantonensis  
Angiostrongylus\_costaricensis  
Anisakis\_simplex  
Annalicia\_algerae  
Anolis\_carolinensis  
Anopheles\_albimanus  
Anopheles\_arabiensis  
Anopheles\_atroparvus  
Anopheles\_christyi  
Anopheles\_coluzzii  
Anopheles\_culicifacies  
Anopheles\_darlingi  
Anopheles\_dirus  
Anopheles\_epiroticus  
Anopheles\_farauti  
Anopheles\_funestus  
Anopheles\_gambiae  
Anopheles\_maculatus  
Anopheles\_melas  
Anopheles\_merus  
Anopheles\_minimus  
Anopheles\_quadriannulatus  
Anopheles\_sinensis  
Anopheles\_stephensi  
Antirrhinum\_majus  
Apis\_mellifera  
Aplysia\_californica  
Aptenodytes\_forsteri  
Aquilegia\_coerulea  
Arabidopsis\_lyrata  
Arabidopsis\_thaliana  
Artemia\_franciscana  
Arthrobotrys\_oligospora  
Arthroderma\_benhamiae  
Arthroderma\_otae  
Ascaris\_lumbricoides  
Ascaris\_suum  
Ashbya\_gossypii  
Aspergillus\_carbonarius  
Aspergillus\_clavatus  
Aspergillus\_flavus  
Aspergillus\_fumigatus  
Aspergillus\_nidulans  
Aspergillus\_niger  
Aspergillus\_oryzae  
Aspergillus\_ruber  
Aspergillus\_terreus  
Astyanax\_mexicanus  
Atta\_cephalotes  
Aureobasidium\_melanogenum  
Aureobasidium\_pullulans  
Aureobasidium\_subglaciale  
Aureococcus\_anophagefferens  
Avian\_erythroblastosis\_virus

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Avian\_musculoaponeurotic\_fibrosarcoma\_virus\_AS42  
Avian\_myeloblastosis\_virus  
Avian\_sarcoma\_virus\_17  
Babesia\_bovis  
Batrachochytrium\_dendrobatidis  
Baudoinia\_compniacensis  
Beauveria\_bassiana  
Bigelowiella\_natans  
Biomphalaria\_glabrata  
Bipolaris\_maydis  
Bipolaris\_oryzae  
Bipolaris\_sorokiniana  
Bipolaris\_victoriae  
Bipolaris\_zeicola  
Blastomyces\_dermatitidis  
Blumeria\_graminis  
Boechera\_stricta  
Bombyx\_mori  
Bos\_grunniens  
Bos\_taurus  
Botryobasidium\_botryosum  
Botrytis\_cinerea  
Bovine\_papillomavirus\_type\_2  
Brachypodium\_distachyon  
Branchiostoma\_floridae  
Brassica\_napus  
Brassica\_oleracea  
Brassica\_rapa  
Brettanomyces\_bruxellensis  
Brugia\_malayi  
Brugia\_pahangi  
Brugia\_timori  
Buceros\_rhinoceros  
Bursaphelenchus\_xylophilus  
Byssochlamys\_spectabilis  
Caenorhabditis\_brenneri  
Caenorhabditis briggsae  
Caenorhabditis\_elegans  
Caenorhabditis\_japonica  
Caenorhabditis\_remanei  
Callithrix\_jacchus  
Calypte\_anna  
Camponotus\_floridanus  
Candida\_albicans  
Candida\_dubliniensis  
Candida\_glabrata  
Candida\_guilliermondii  
Candida\_lusitaniae  
Candida\_maltosa  
Candida\_orthopsilosis  
Candida\_parapsilosis  
Candida\_tenuis  
Candida\_tropicalis  
Canis\_familiaris  
Cannabis\_sativa  
Capitella\_teleta  
Capronia\_coronata

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Capronia\_epimyces  
Capronia\_semiimmersa  
Capsaspora\_owczarzaki  
Capsella\_grandiflora  
Capsella\_rubella  
Carica\_papaya  
Cavia\_porcellus  
Ceriporiopsis\_subvermispora  
Chaetomium\_globosum  
Chaetomium\_thermophilum  
Chelonia\_mydas  
Chlamydomonas\_reinhardtii  
Chlorella\_NC64A  
Chlorella\_vulgaris  
Chlorocebus\_sabaeus  
Choloepus\_hoffmanni  
Chroomonas\_mesostigmatica  
Cicer\_arietinum  
Cimex\_lectularius  
Ciona\_intestinalis  
Ciona\_savignyi  
Citrullus\_lanatus  
Citrus\_clementina  
Citrus\_sinensis  
Cladophialophora\_bantiana  
Cladophialophora\_carrionii  
Cladophialophora\_immundia  
Cladophialophora\_psammophila  
Cladophialophora\_yegresii  
Claviceps\_purpurea  
Clavispore\_lusitaniae  
Clonorchis\_sinensis  
Coccidioides\_immitis  
Coccidioides\_posadasii  
Coccomyxa\_subellipsoidea\_C169  
Cochliobolus\_heterostrophus\_C5  
Colletotrichum\_fioriniae  
Colletotrichum\_gloeosporioides  
Colletotrichum\_graminicola  
Colletotrichum\_higginsianum  
Colletotrichum\_orbiculare  
Colletotrichum\_sublineola  
Coniosporium\_apollinis  
Coprinellus\_disseminatus  
Coprinopsis\_cinerea  
Coprinopsis\_scobicola  
Cordyceps\_militaris  
Crassostrea\_gigas  
Cryphonectria\_parasitica  
Cryptococcus\_gattii  
Cryptococcus\_neoformans  
Cryptosporidium\_hominis  
Cryptosporidium\_muris  
Cryptosporidium\_parvum  
Cucumis\_sativus  
Culex\_pipiens  
Culex\_quinquefasciatus

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Cupiennius\_salei  
Cyanidioschyzon\_merolae  
Cyclicostephanus\_goldi  
Cyphelophora\_europaea  
Dacryopinax\_sp  
Dactylellina\_haptotyla  
Danaus\_plexippus  
Danio\_rerio  
Daphnia\_pulex  
Dasypus\_novemcinctus  
Debaryomyces\_hansenii  
Dendroctonus\_ponderosae  
Dichomitus\_squalens  
Dictyocaulus\_viviparus  
Dictyostelium\_discoideum  
Dictyostelium\_purpureum  
Diphyllobothrium\_latum  
Dipodomys\_ordii  
Dirofilaria\_immitis  
Discocelis\_tigrina  
Dothistroma\_septosporum  
Dracunculus\_medinensis  
Drechslerella\_stenobrocha  
Drosophila\_ananassae  
Drosophila\_erecta  
Drosophila\_grimshawi  
Drosophila\_melanogaster  
Drosophila\_mojavensis  
Drosophila\_persimilis  
Drosophila\_pseudoobscura  
Drosophila\_sechellia  
Drosophila\_simulans  
Drosophila\_virilis  
Drosophila\_willistoni  
Drosophila\_yakuba  
Echinococcus\_canadensis  
Echinococcus\_granulosus  
Echinococcus\_multilocularis  
Echinops\_telfairi  
Echinostoma\_caproni  
Edhazardia\_aedis  
Eimeria\_tenella  
Elaeophora\_elaphi  
Eleutheria\_dichotoma  
Emiliania\_huxleyi  
Encephalitozoon\_cuniculi  
Encephalitozoon\_hellem  
Encephalitozoon\_intestinalis  
Encephalitozoon\_romaleae  
Endocarpon\_pusillum  
Entamoeba\_dispar  
Entamoeba\_histolytica  
Entamoeba\_invadens  
Enterobius\_vermicularis  
Enterocytozoon\_bieneusi  
EpsteinBarr\_virus  
Equus\_caballus

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Eremothecium\_cymbalariae  
Erinaceus\_europaeus  
Erysiphe\_necator  
Eucalyptus\_grandis  
Eutrema\_salsugineum  
Eutypa\_lata  
Exophiala\_aquamarina  
Exophiala\_dermatitidis  
Exophiala\_mesophila  
Exophiala\_oligosperma  
Exophiala\_sideris  
Exophiala\_spinifera  
Exophiala\_xenobiotica  
Fasciola\_hepatica  
Felis\_catus  
Fibroporia\_radiculosa  
Ficedula\_albicollis  
Fomitopsis\_pinicola  
Fonsecaea\_pedrosoi  
Fragaria Vesca  
Fragilariopsis\_cylindrus  
Fusarium\_fujikuroi  
Fusarium\_graminearum  
Fusarium\_oxysporum  
Fusarium\_pseudograminearum  
Fusarium\_solani  
Fusarium\_verticillioides  
Gadus\_morhua  
Gaeumannomyces\_graminis  
Galerina\_marginata  
Gallid herpesvirus\_2  
Gallus\_gallus  
Gasterosteus\_aculeatus  
Geospiza\_fortis  
Giardia\_lamblia  
Glarea\_lozoyensis  
Globodera\_pallida  
Gloeophyllum\_trabeum  
Glycine\_max  
Gongylonema\_pulchrum  
Gorilla\_gorilla  
Gossypium\_raimondii  
Grosmannia\_clavigera  
Guillardia\_theta  
Gymnoporus\_luxurians  
Haemonchus\_contortus  
Haemonchus\_placei  
Halocynthia\_roretzii  
Halyomorpha\_halys  
Harpegnathos\_saltator  
Hebeloma\_cylindrospororum  
Helianthus\_annuus  
Heliconius\_melpomene  
Heligmosomoides\_bakeri  
Helobdella\_robusta  
Hemiselmis\_andersenii  
Heterobasidion\_annosum

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Heterobasidion\_irregulare  
 Heterodontus\_francisci  
 Heterorhabditis\_bacteriophora  
 Histoplasma\_capsulatum  
 Homo\_sapiens  
 Hordeum\_vulgare  
 Hyaloperonospora\_arabidopsisidis  
 Hydatigera\_taeniaeformis  
 Hydnomerulius\_pinastri  
 Hydra\_magnipapillata  
 Hymenolepis\_diminuta  
 Hymenolepis\_microstoma  
 Hymenolepis\_nana  
 Ictidomys\_tridecemlineatus  
 Ipomoea\_batatas  
 Ixodes\_scapularis  
 Jaapia\_argillacea  
 Kazachstania\_africana  
 Kazachstania\_naganishii  
 Kluyveromyces\_delphensis  
 Kluyveromyces\_lactis  
 Kluyveromyces\_thermotolerans  
 Kluyveromyces\_waltii  
 Komagataella\_pastoris  
 Kuraishia\_capsulata  
 Laccaria\_amethystina  
 Laccaria\_bicolor  
 Lachancea\_kluyveri  
 Lachancea\_lanzarotensis  
 Lachancea\_thermotolerans  
 Latimeria\_chalumnae  
 Leersia\_perrieri  
 Leishmania\_braziliensis  
 Leishmania\_infantum  
 Leishmania\_major  
 Leishmania\_mexicana  
 Lepisosteus\_oculatus  
 Leptinotarsa\_decemlineata  
 Leptosphaeria\_maculans  
 Lingula\_unguis  
 Linum\_usitatissimum  
 Litomosoides\_sigmodontis  
 Loa\_loa  
 Lodderomyces\_elongisporus  
 Lottia\_gigantea  
 Lotus\_japonicus  
 Loxodonta\_africana  
 Lucilia\_cuprina  
 Lutzomyia\_longipalpis  
 Lycopersicon\_esculentum  
 Macaca\_fascicularis  
 Macaca\_mulatta  
 Macrohomina\_phaseolina  
 Macropus\_eugenii  
 Magnaporthe\_oryzae  
 Magnaporthe\_poae  
 Malassezia\_globosa

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Malassezia\_sympodialis  
Malus\_domestica  
Manihot\_esculenta  
Marmota\_monax  
Marssonina\_brunnea  
Medicago\_sativa  
Medicago\_truncatula  
Megasselia\_scalaris  
Melampsora\_laricipopulina  
Meleagris\_gallopavo  
Melitaea\_cinxia  
Meloidogyne\_floridensis  
Meloidogyne\_hapla  
Meloidogyne\_incognita  
Mesocestoides\_corti  
Metarhizium\_acridum  
Metarhizium\_album  
Metarhizium\_anisopliae  
Metarhizium\_brunneum  
Metarhizium\_guizhouense  
Metarhizium\_majus  
Metarhizium\_robertsii  
Meyerozyma\_guilliermondii  
Microbotryum\_violaceum  
Microcebus\_murinus  
Micromonas\_pusilla  
Micromonas\_sp\_RCC299  
Microsporidia\_sp  
Microsporum\_canis  
Microsporum\_gypseum  
Millerozyma\_farinosa  
Mimulus\_guttatus  
Mixia\_osmundae  
Mnemiopsis\_leidyi  
Moniliophthora\_perniciosa  
Moniliophthora\_roreri  
Monodelphis\_domestica  
Monosiga\_brevicollis  
Mucor\_circinelloides  
Mus\_musculus  
Musa\_acuminata  
Musca\_domestica  
Mustela\_putorius\_furo  
Myceliophthora\_thermophila  
Mycosphaerella\_fijiensis  
Mycosphaerella\_graminicola  
Myotis\_brandtii  
Myotis\_lucifugus  
Naegleria\_gruberi  
Nasonia\_vitripennis  
Naumovozyma\_castellii  
Naumovozyma\_dairenensis  
Necator\_americanus  
Nectria\_haematoxocca  
Nematocida\_parisi\_ertm1  
Nematocida\_sp  
Nematostella\_vectensis

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Neofusicoccum\_parvum  
 Neosartorya\_fischeri  
 Neospora\_caninum  
 Neurospora\_crassa  
 Neurospora\_discreta  
 Neurospora\_tetrasperma  
 Nicotiana\_sp.  
 Nicotiana\_tabacum  
 Nippostrongylus\_brasiliensis  
 Nomascus\_leucogenys  
 Nosema\_apis  
 Nosema\_bombycis  
 Nosema\_ceranae  
 Ochotona\_princeps  
 Octopus\_bimaculoides  
 Oesophagostomum\_dentatum  
 Ogataea\_parapolymorpha  
 Oidiodendron\_maius  
 Oikopleura\_dioica  
 Onchocerca\_flexuosa  
 Onchocerca\_ochengi  
 Onchocerca\_volvulus  
 Oncorhynchus\_tshawytscha  
 Ophiocordyceps\_sinensis  
 Ophiostoma\_piceae\_uamh  
 Ophisaurus\_gracilis  
 Opisthorchis\_viverrini  
 Ordospora\_colligata  
 Oreochromis\_niloticus  
 Ornithorhynchus\_anatinus  
 Oryctolagus\_cuniculus  
 Oryza\_barthii  
 Oryza\_brachyantha  
 Oryza\_glaberrima  
 Oryza\_glumaepatula  
 Oryza\_indica  
 Oryza\_longistaminata  
 Oryza\_meridionalis  
 Oryza\_nivara  
 Oryza\_punctata  
 Oryza\_rufipogon  
 Oryza\_sativa  
 Oryzias\_latipes  
 Ostreococcus\_RCC809  
 Ostreococcus\_lucimarinus  
 Ostreococcus\_tauri  
 Otolemur\_garnettii  
 Ovis\_aries  
 Oxytricha\_trifallax  
 PBM\_CONSTRUCTS  
 Pan\_paniscus  
 Pan\_troglodytes  
 Panicum\_virgatum  
 Papio\_anubis  
 Paracoccidioides\_brasiliensis  
 Paracoccidioides\_sp\_lutzii  
 Paramecium\_tetraurelia

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Parascaris\_equorum  
Parastrongyloides\_trichosuri  
Patiria\_miniata  
Paxillus\_involutus  
Paxillus\_rubicundulus  
Pediculus\_humanus  
Pelodiscus\_sinensis  
Penicillium\_chrysogenum  
Penicillium\_digitatum  
Penicillium\_expansum  
Penicillium\_italicum  
Penicillium\_marneffei  
Penicillium\_oxalicum  
Penicillium\_rubens  
Penicillium\_solitum  
Pestalotiopsis\_fici  
Petromyzon\_marinus  
Petroselinum\_crispum  
Petunia\_x\_hybrida  
Phaeodactylum\_tricornutum  
Phaeosphaeria\_nodorum  
Phanerochaete\_carnosa  
Phanerochaete\_chrysosporium  
Phaseolus\_vulgaris  
Phlebiopsis\_gigantea  
Phlebotomus\_papatasi  
Phoenix\_dactylifera  
Phycomyces\_blakesleeanus  
Physcomitrella\_patens  
Phytophthora\_capsici  
Phytophthora\_infestans  
Phytophthora\_kernoviae  
Phytophthora\_lateralis  
Phytophthora\_parasitica  
Phytophthora\_ramorum  
Phytophthora\_sojae  
Pichia\_angusta  
Pichia\_kudriavzevii  
Pichia\_pastoris  
Pichia\_stipitis  
Piloderma\_croceum  
Piriformospora\_indica  
Pisolithus\_microcarpus  
Pisolithus\_tinctorius  
Pisum\_sativum  
Plasmodium\_berghei  
Plasmodium\_chabaudi  
Plasmodium\_falciparum  
Plasmodium\_knowlesi  
Plasmodium\_vivax  
Plasmodium\_yoelii  
Pleurobrachia\_pileus  
Pleurotus\_djamor  
Pleurotus\_ostreatus  
Plicaturopsis\_crispa  
Pneumocystis\_carinii  
Pneumocystis\_murina

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Podocoryne\_carnea  
 Podospora\_anserina  
 Poecilia\_formosa  
 Pogona\_vitticeps  
 Polysphondylium\_pallidum  
 Pongo\_abelii  
 Populus\_trichocarpa  
 Postia\_placenta  
 Pristionchus\_exspectatus  
 Pristionchus\_pacificus  
 Procavia\_capensis  
 Protopolystoma\_xenopodis  
 Prunus\_mume  
 Prunus\_persica  
 Pseudocercospora\_fijiensis  
 Pseudogymnoascus\_destructans  
 Pseudogymnoascus\_pannorum  
 Pseudozyma\_antarctica  
 Pseudozyma\_aphidis  
 Pseudozyma\_brasiliensis  
 Pseudozyma\_flocculosa  
 Pseudozyma\_hubeiensis  
 Pteropus\_vampyrus  
 Puccinia\_graminis  
 Puccinia\_triticina  
 Punctularia\_strigosozonata  
 Pyrenophora\_teres  
 Pyrenophora\_triticirepentis  
 Pythium\_aphanidermatum  
 Pythium\_arrhenomanes  
 Pythium\_irregulare  
 Pythium\_iwayamai  
 Pythium\_ultimum  
 Pythium\_vexans  
 Rattus\_norvegicus  
 Rhabditophanes\_kr3021  
 Rhinocladiella\_mackenziei  
 Rhizoctonia\_solani  
 Rhizophagus\_irregularis  
 Rhizopus\_oryzae  
 Rhodnius\_prolixus  
 Rhodosporidium\_toruloides  
 Rhodotorula\_glutinis  
 Ricinus\_communis  
 Romanomermis\_culicivorax  
 Rozella\_allomycis  
 Saccharomyces\_arboricola  
 Saccharomyces\_bayanus  
 Saccharomyces\_castellii  
 Saccharomyces\_cerevisiae  
 Saccharomyces\_kudriavzevii  
 Saccharomyces\_mikatae  
 Saccharomyces\_paradoxus  
 Saccharomyctaceae\_sp\_ashbya\_aceri  
 Saccoglossus\_kowalevskii  
 Salix\_purpurea  
 Salpingoeca\_rosetta

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Saprolegnia\_parasitica  
Sarcophilus\_harrisii  
Sarsia\_sp.\_Long\_Island\_Sound  
Scedosporium\_apiospermum  
Scheffersomyces\_stipitis  
Schistocephalus\_solidus  
Schistosoma\_curassoni  
Schistosoma\_haematobium  
Schistosoma\_japonicum  
Schistosoma\_mansoni  
Schistosoma\_margrebowiei  
Schistosoma\_mattheei  
Schistosoma\_rodhaini  
Schizophyllum\_commune  
Schizosaccharomyces\_cryophilus  
Schizosaccharomyces\_japonicus  
Schizosaccharomyces\_octosporus  
Schizosaccharomyces\_pombe  
Schmidtea\_mediterranea  
Scleroderma\_citrinum  
Sclerotinia\_borealis  
Sclerotinia\_sclerotiorum  
Selaginella\_moellendorffii  
Serendipita\_vermifera  
Serpula\_lacrymans  
Setaria\_italica  
Setosphaeria\_turcica  
Soboliphyme\_baturini  
Solanum\_lycopersicum  
Solanum\_tuberosum  
Solenopsis\_invicta  
Sordaria\_macrospora  
Sorex\_araneus  
Sorghum\_bicolor  
Spadella\_cephaloptera  
Spathaspora\_passalidarum  
Sphaerobolus\_stellatus  
Sphaerulina\_musiva  
Spirometra\_erinaceieuropaei  
Spizellomyces\_punctatus  
Sporisorium\_reilianum  
Sporobolomyces\_roseus  
Sporothrix\_brasiliensis  
Sporothrix\_schenckii  
Spraguea\_lophii  
Stachybotrys\_chartarum  
Stachybotrys\_chlorohalonata  
Stagonospora\_nodorum  
Steinernema\_carpocapsae  
Steinernema\_feltiae  
Steinernema\_glaseri  
Steinernema\_monticolum  
Steinernema\_scapterisci  
Strigamia\_maritima  
Strongylocentrotus\_purpuratus  
Strongyloides\_papillosus  
Strongyloides\_stercoralis

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Strongyloides\_venezuelensis  
Strongylus\_vulgaris  
Suillus\_luteus  
Sus\_scrofa  
Syphacia\_muris  
Taenia\_asiatica  
Taenia\_solium  
Taeniopygia\_guttata  
Takifugu\_rubripes  
Talaromyces\_marneffei  
Talaromyces\_stipitatus  
Tarsius\_syrichta  
Teladorsagia\_circumcincta  
Tetrahymena\_thermophila  
Tetranychus\_urticae  
Tetraodon\_nigroviridis  
Tetrapisispora\_blaetae  
Tetrapisispora\_phaffii  
Thalassiosira\_pseudonana  
Thecamonas\_trahens  
Theileria\_annulata  
Theileria\_parva  
Thelazia\_callipaeda  
Theobroma\_cacao  
Thielavia\_terrestris  
Tilletiaria\_anomala  
Togninia\_minima  
Torrubiella\_hemipterigena  
Torulaspora\_delbrueckii  
Toxocara\_canis  
Toxoplasma\_gondii  
Trachipleistophora\_hominis  
Trametes\_cinnabarina  
Tremella\_mesenterica  
Tribolium\_castaneum  
Trichinella\_nativa  
Trichinella\_spiralis  
Trichobilharzia\_regenti  
Trichoderma\_atroviride  
Trichoderma\_reesei  
Trichoderma\_virens  
Trichomonas\_vaginalis  
Trichophyton\_equinum  
Trichophyton\_interdigitale  
Trichophyton\_rubrum  
Trichophyton\_soudanense  
Trichophyton\_tonsurans  
Trichophyton\_verrucosum  
Trichoplax\_adhaerens  
Trichosporon\_asahii  
Trichuris\_muris  
Trichuris\_suis  
Trichuris\_trichiura  
Trionyx\_sinensis  
Tripedalia\_cystophora  
Triticum\_aestivum  
Triticum\_urartu

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```
Trypanosoma_brucei
Trypanosoma_congolense
Trypanosoma_cruzi
Trypanosoma_vivax
Tuber_melanosporum
Tulasnella_calospora
Tupaia_belangeri
Tursiops_truncatus
Tyto_alba
Uncinocarpus_reesii
Ustilaginoidea_virens
Ustilago_hordei
Ustilago_maydis
Vanderwaltozyma_polyspora
Vavraia_culicis
Verruconis_gallopava
Verticillium_albo_atrum
Verticillium_alfalfa
Verticillium_dahliae
Vicugna_pacos
Vitis_vinifera
Vittaforma_corneae
Volvox_carteri
Wallemia_ichthyophaga
Wallemia_sebi
Wickerhamomyces_ciferrii
Wuchereria_bancrofti
Xenopus_laevis
Xenopus_tropicalis
Xiphophorus_maculatus
Yarrowia_lipolytica
Zea_mays
Zootermopsis_nevadensis
Zygosaccharomyces_bailii
Zygosaccharomyces_rouxii
Zymoseptoria_tritici
```

```
[18]: # Pick up motif information for one species and save as gummemotif pfm file format.

species = "Danio_rerio"
make_motif_file_from_cisbp_data(pwm_folder_path="pwms", tfinfo_df=df, species=species)
(6133,)
(109560, 4) 5298
```

#### 4. Check results

```
[19]: # Read motifs

from gummemotifs.motif import read_motifs

path = f"CisDB_ver2_{species}.pfm"
custom_motifs = read_motifs(path)
custom_motifs[:10]
```

```
[19]: [M00008_2.00_nnnAAww,
M00045_2.00_GTAAACAA,
M00056_2.00_TAATAAAT,
M00066_2.00_nsGTTGCyAn,
M00070_2.00_nrAACAAATAnn,
M00111_2.00_nGCCynnGGs,
M00112_2.00_CCTsrGGCnA,
M00113_2.00_nsCCnnAGGs,
M00114_2.00_nnGCCynnGG,
M00115_2.00_nnATnAAAn]
```

```
[ ]: # Delete downloaded data
```

```
[1]: ! rm -r TF_Information_all_motifs*
! rm PWMs.*
! rm -r pwms
```

### 1.2.3 Single-cell RNA-seq data preprocessing

Network analysis and simulation in celloracle will be performed using scRNA-seq data. The scRNA-seq data should include the components below.

- Gene expression matrix; mRNA counts before scaling and transformation.
- Clustering results.
- Dimensional reduction results.

In addition to these minimum requirements, we highly recommend doing these analyses below in the preprocessing step.

- Data quality check and cell/gene filtering.
- Normalization
- Identification of highly variable genes

We recommend processing scRNA-seq data using either Scanpy or Seurat. If you are not familiar with the general workflow of scRNA-seq data processing, please go to [the documentation for scanpy](#) and [the documentation for Seurat](#) before celloracle analysis.

If you already have preprocessed scRNA-seq data, which includes the necessary information above, you can skip this part.

#### A. scRNA-seq data preprocessing with scanpy

scanpy is a python library for the analysis of scRNA-seq data.

In this tutorial, we introduce an example of scRNA-seq preprocessing for celloracle with `scanpy`. We wrote the notebook based on [one of scanpy's tutorials](#) with some modifications.

The jupyter notebook files and data used in this tutorial are available [here](#).

[Python notebook](#)

## 0. Import libraries

```
[1]: import os
import matplotlib.pyplot as plt
import numpy as np
import pandas as pd
import scanpy as sc
```

```
[2]: %matplotlib inline
%config InlineBackend.figure_format = 'retina'
plt.rcParams["savefig.dpi"] = 300
plt.rcParams["figure.figsize"] = [6, 4.5]
```

## 1. Load data

In this notebook, we will show an example of how to process scRNA-seq data using a scRNA-seq data of hematopoiesis (Paul, F., Arkin, Y., Giladi, A., Jaitin, D. A., Kenigsberg, E., Keren-Shaul, H., et al. (2015). Transcriptional Heterogeneity and Lineage Commitment in Myeloid Progenitors. *Cell*, 163(7), 1663–1677. <http://doi.org/10.1016/j.cell.2015.11.013>). You can easily download this scRNA-seq data with a `scanpy` function.

Please change the code below if you want to use your data.

```
[3]: # Download dataset. You can change the code blow if you use another data.
adata = sc.datasets.paul15()
```

WARNING: In Scanpy 0.\*, this returned logarithmized data. Now it returns non-  
logarithmized data.

```
... storing 'paul15_clusters' as categorical
Trying to set attribute `uns` of view, making a copy.
```

## 2. Filtering

```
[4]: # Only consider genes with more than 1 count
sc.pp.filter_genes(adata, min_counts=1)
```

## 3. Normalization

```
[5]: # Normalize gene expression matrix with total UMI count per cell
sc.pp.normalize_per_cell(adata, key_n_counts='n_counts_all')
```

## 4. Identification of highly variable genes

Removing non-variable genes not only reduces the calculation time during the GRN reconstruction and simulation, but also improve the accuracy of GRN inference. We recommend using the top 2000~3000 variable genes.

```
[6]: # Select top 2000 highly-variable genes
filter_result = sc.pp.filter_genes_dispersion(adata.X,
                                              flavor='cell_ranger',
                                              n_top_genes=2000,
                                              log=False)

# Subset the genes
adata = adata[:, filter_result.gene_subset]

# Renormalize after filtering
sc.pp.normalize_per_cell(adata)

Trying to set attribute `obs` of view, making a copy.
```

## 5. Log transformation

We will do log transformation scaling because these are necessary for PCA, clustering, and differential gene calculations. However, we also need non-transformed gene expression data in the celloracle analysis. Thus we keep raw count in anndata using the following command before the log transformation.

```
[7]: # keep raw count data before log transformation
adata.raw = adata

# Log transformation and scaling
sc.pp.log1p(adata)
sc.pp.scale(adata)
```

## 6. Dimensional reduction

Dimensional reduction is one of the most important parts of the scRNA-seq analysis. Celloracle needs dimensional reduction embeddings to simulate cell transition.

Please choose a proper algorithm for dimensional reduction so that the embedding appropriately represents the data structure. We recommend using one of these dimensional reduction algorithms (or trajectory inference algorithms); UMAP, tSNE, diffusion map, force-directed graph drawing or PAGA.

In this example, we use a combination of four algorithms; diffusion map, force-directed graph drawing, and PAGA.

```
[9]: # PCA
sc.tl.pca(adata, svd_solver='arpack')

[10]: # Diffusion map
sc.pp.neighbors(adata, n_neighbors=4, n_pcs=20)

sc.tl.diffmap(adata)
# Calculate neighbors again based on diffusionmap
sc.pp.neighbors(adata, n_neighbors=10, use_rep='X_diffmap')
```

## 7. Clustering

```
[11]: sc.tl.louvain(adata, resolution=0.8)
```

### (Optional) Re-calculate Dimensional reduction graph

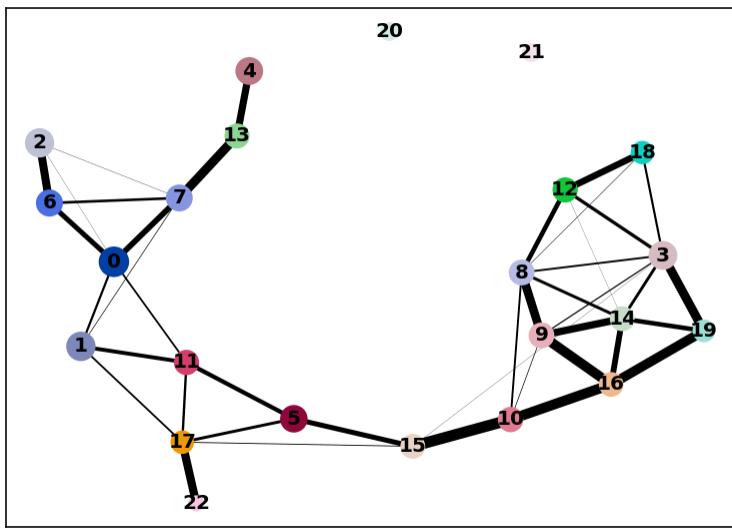
```
[12]: # PAGA graph construction
sc.tl.paga(adata, groups='louvain')
```

```
[13]: # Check current cluster name
cluster_list = adata.obs.louvain.unique()
cluster_list
```

```
[13]: [5, 2, 12, 13, 0, ..., 6, 20, 14, 15, 21]
Length: 23
Categories (23, object): [5, 2, 12, 13, ..., 20, 14, 15, 21]
```

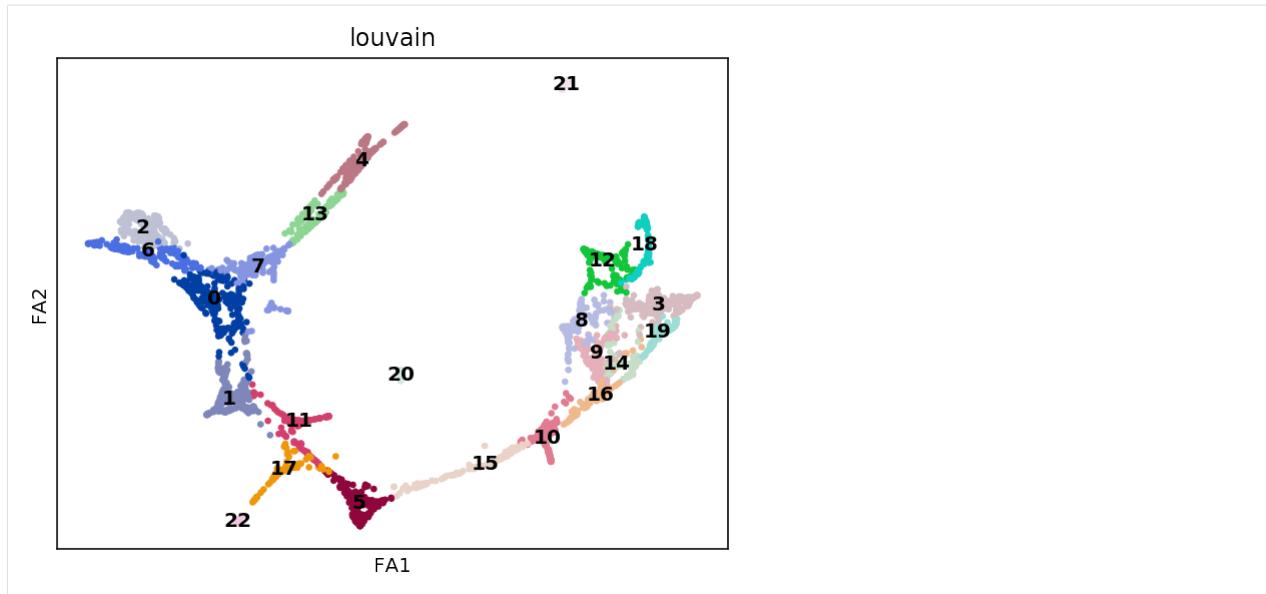
```
[14]: plt.rcParams["figure.figsize"] = [6, 4.5]
```

```
[15]: sc.pl.paga(adata)
```



```
[16]: sc.tl.draw_graph(adata, init_pos='paga', random_state=123)
```

```
[17]: sc.pl.draw_graph(adata, color='louvain', legend_loc='on data')
```



## 8. Check data

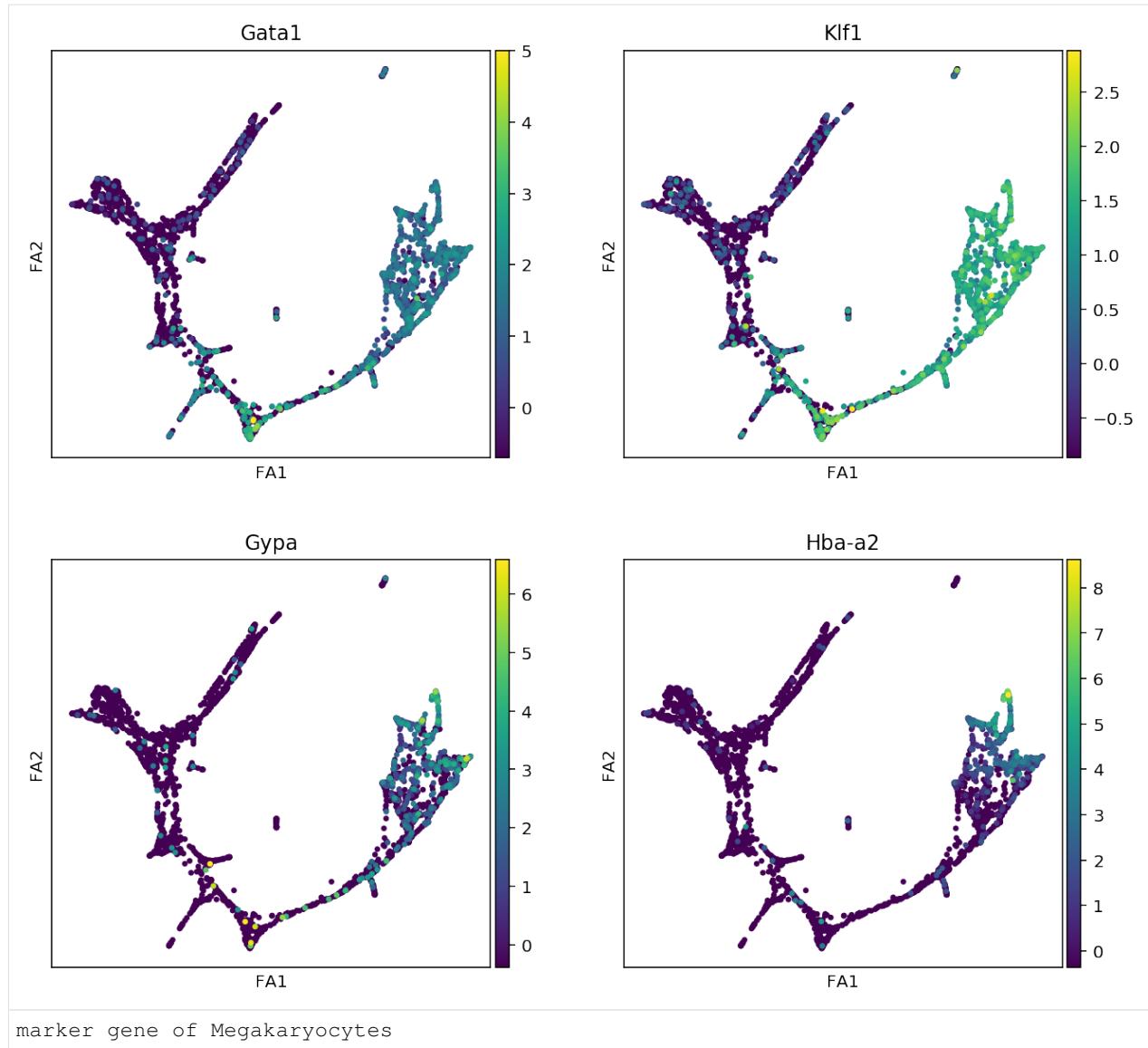
### 8.1. Visualize marker gene expression

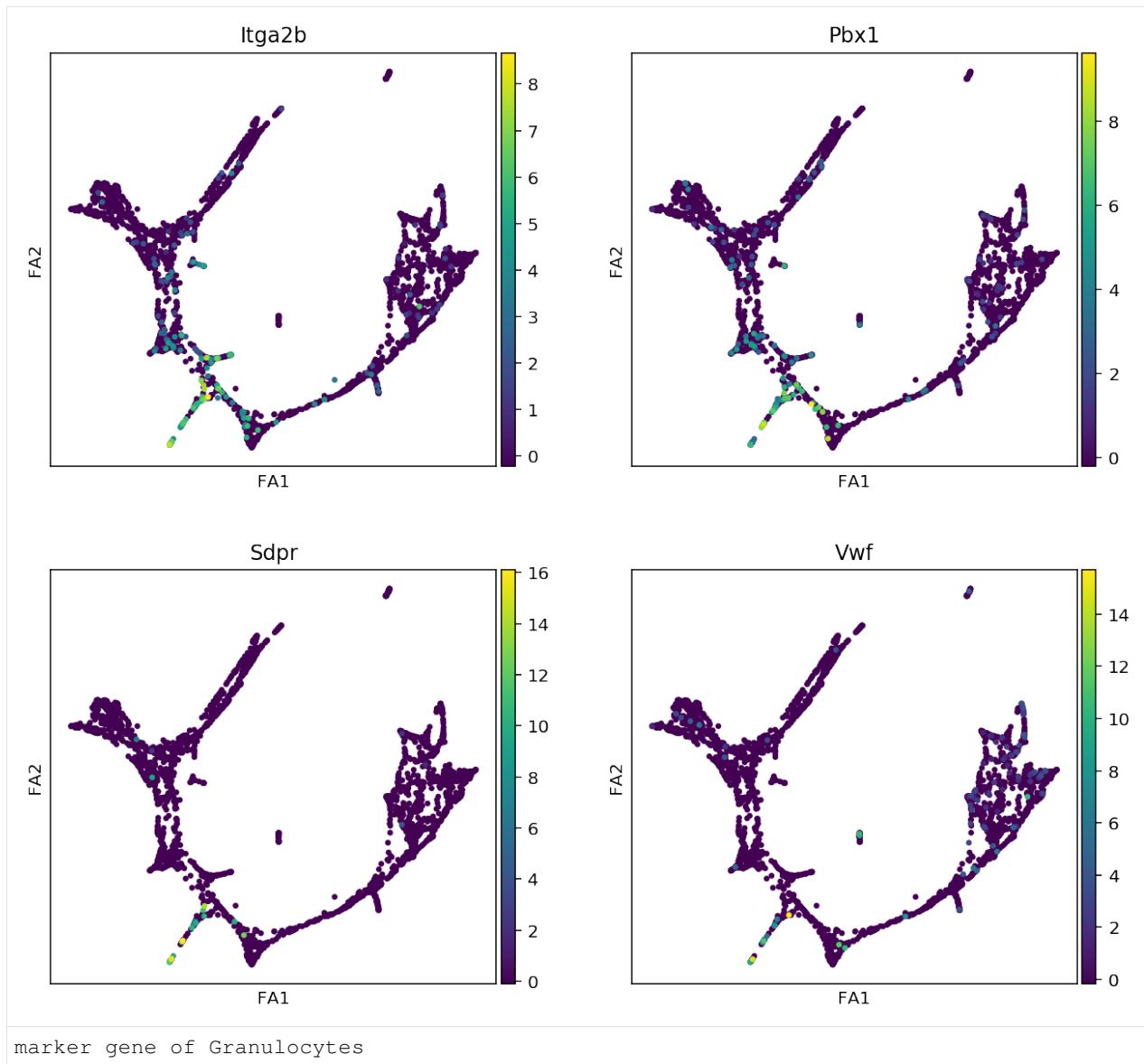
```
[18]: plt.rcParams["figure.figsize"] = [4.5, 4.5]
```

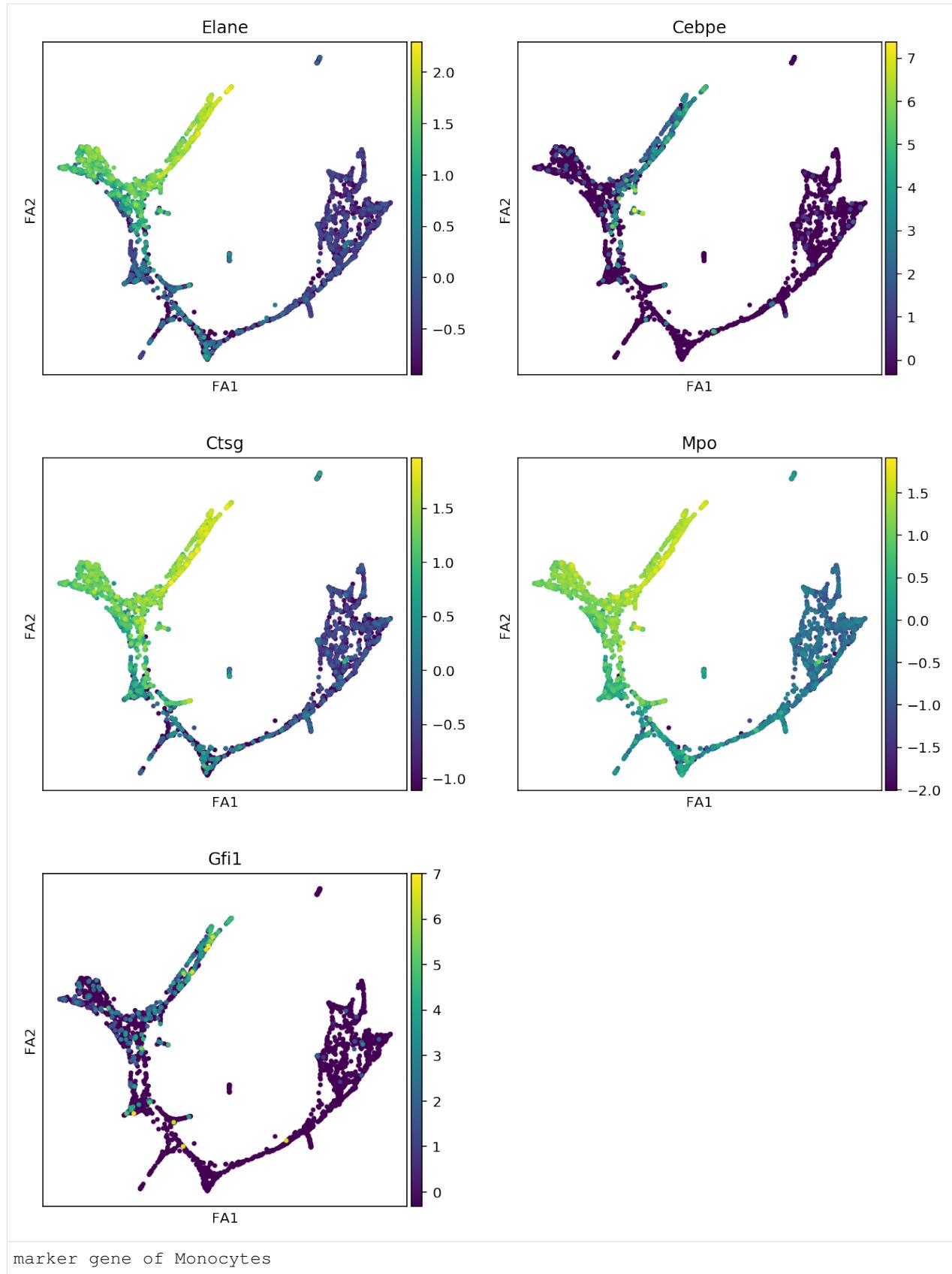
```
[19]: markers = {"Erythroids": ["Gata1", "Klf1", "Gypa", "Hba-a2"],
               "Megakaryocytes": ["Itga2b", "Pbx1", "Sdpr", "Vwf"],
               "Granulocytes": ["Elane", "Cebpe", "Ctsg", "Mpo", "Gfil"],
               "Monocytes": ["Irf8", "Csflr", "Ctsg", "Mpo"],
               "Mast_cells": ["Cma1", "Gzmb", "Kit"],
               "Basophils": ["Mcpt8", "Prss34"]
              }

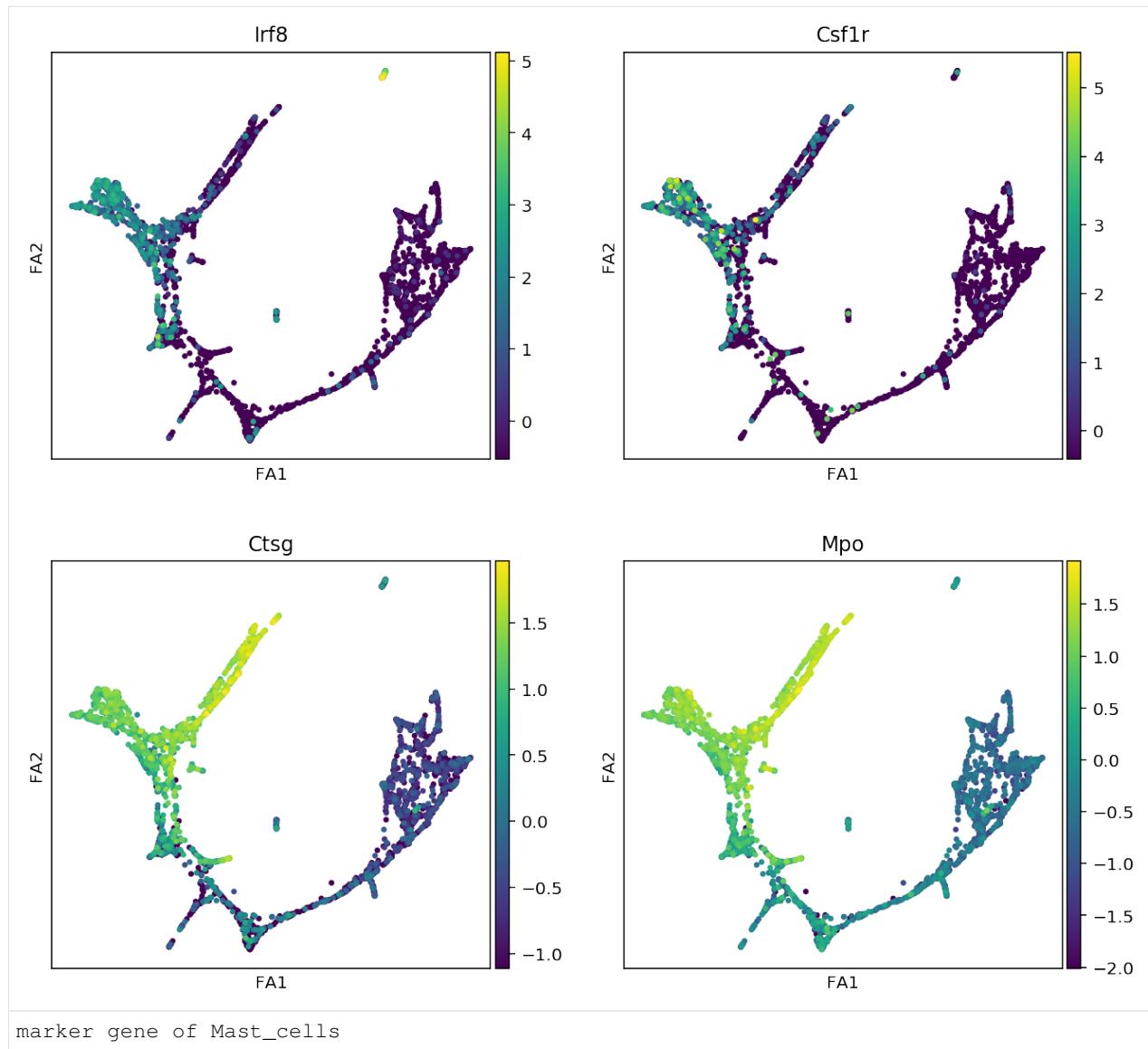
for cell_type, genes in markers.items():
    print(f"marker gene of {cell_type}")
    sc.pl.draw_graph(adata, color=genes, use_raw=False, ncols=2)
    plt.show()
```

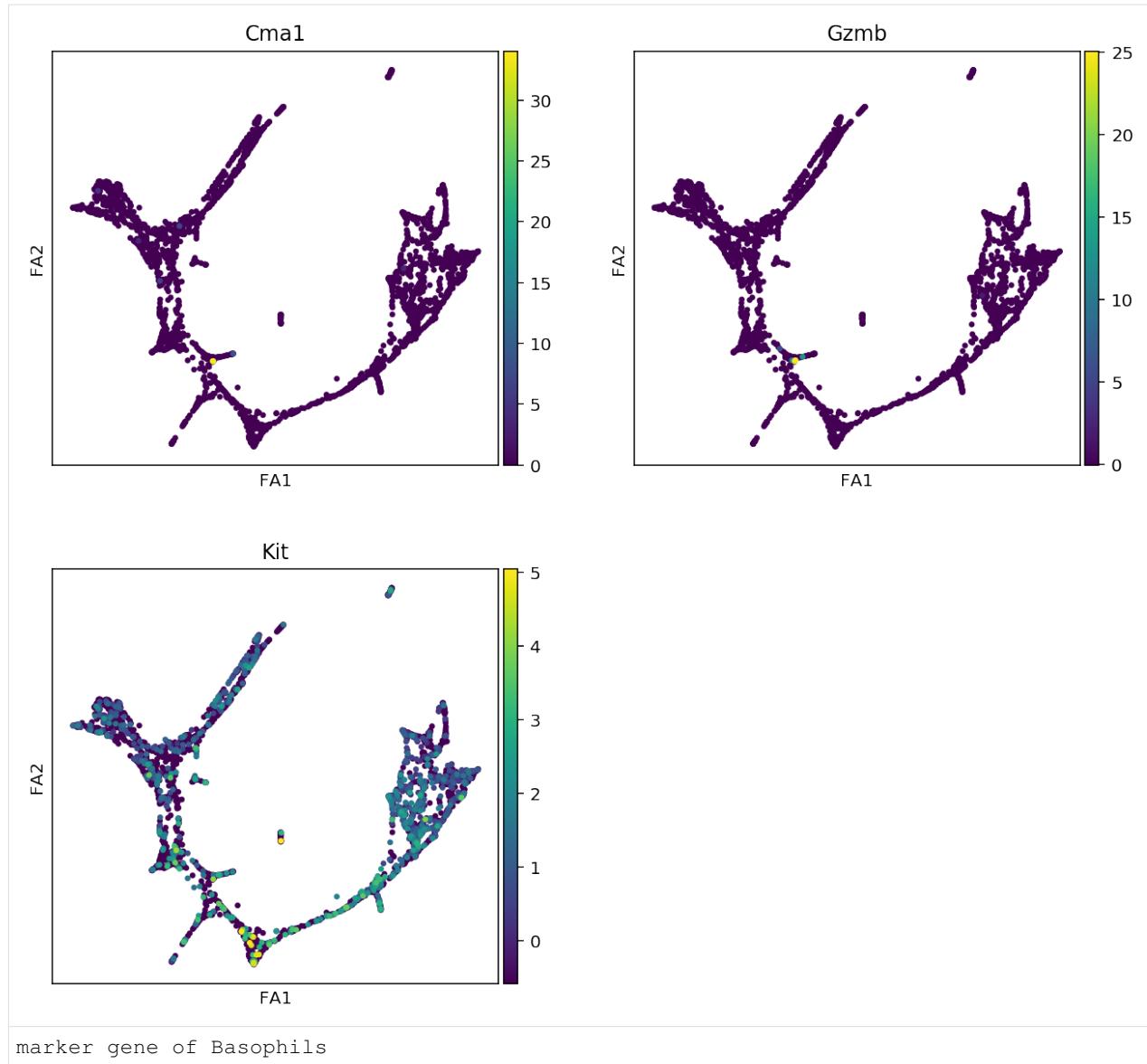
```
marker gene of Erythroids
```

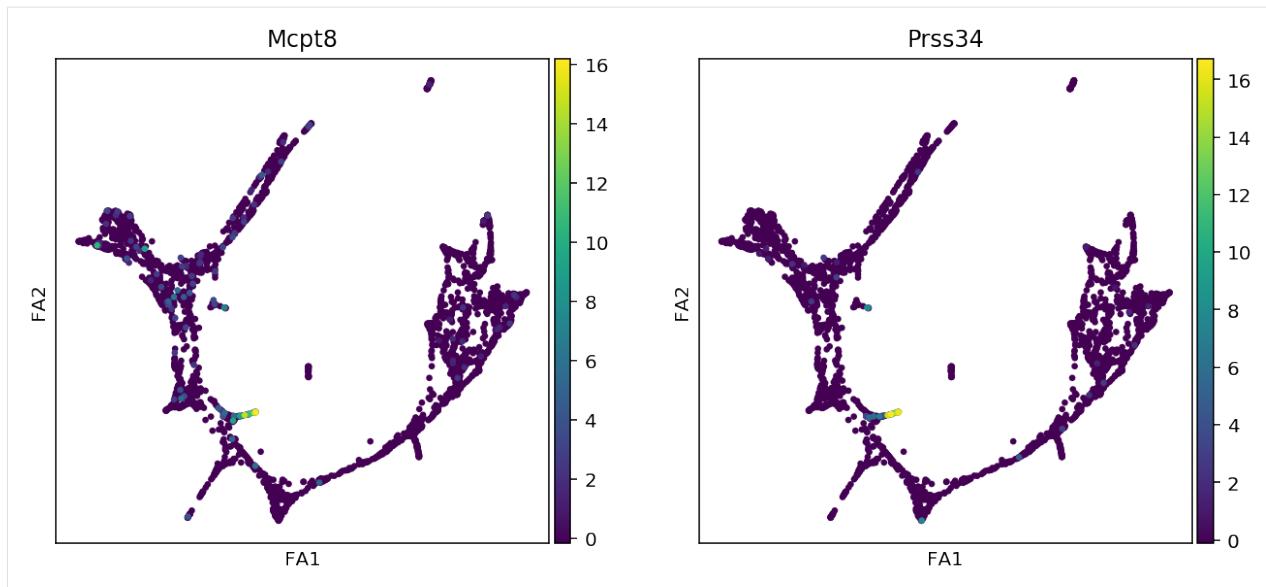










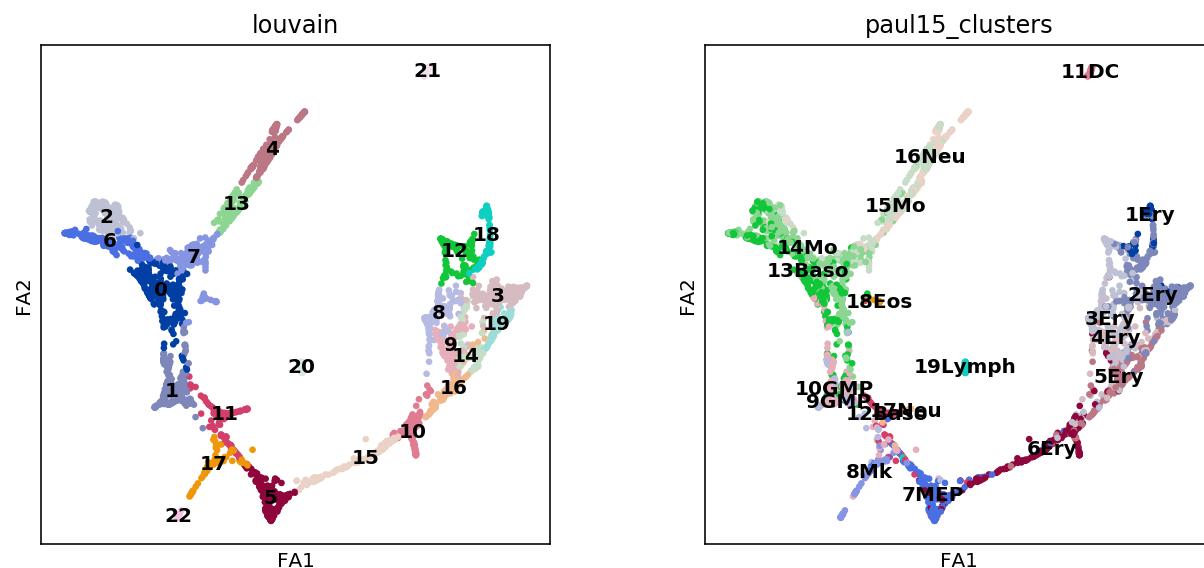


## 9. Make annotation for cluster

Based on the marker gene expression and previous reports, we will manually annotate each cluster. When using your own data, you will need to annotate the clusters appropriately.

### 9.1. Make annotation (1)

```
[20]: sc.pl.draw_graph(adata, color=['louvain', 'paul15_clusters'],
                      legend_loc='on data')
```



```
[21]: # Check current cluster name
cluster_list = adata.obs.louvain.unique()
cluster_list
```

```
[21]: [5, 2, 12, 13, 0, ..., 6, 20, 14, 15, 21]
Length: 23
Categories (23, object): [5, 2, 12, 13, ..., 20, 14, 15, 21]
```

**!! Please change the dictionary below depending on the clustering results. The results may change depending on the execution environment.**

```
[22]: # Make annotation dictionary
annotation = {"MEP": [5],
              "Erythroids": [15, 10, 16, 9, 8, 14, 19, 3, 12, 18],
              "Megakaryocytes": [17, 22],
              "GMP": [11, 1],
              "late_GMP": [0],
              "Granulocytes": [7, 13, 4],
              "Monocytes": [6, 2],
              "DC": [21],
              "Lymphoid": [20]}

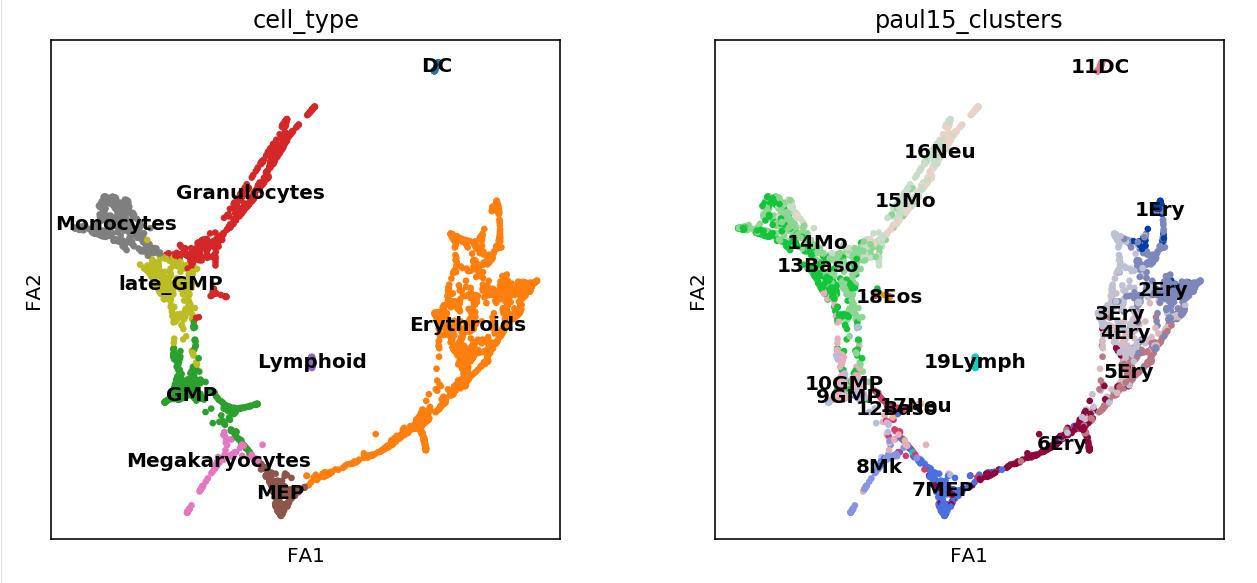
# change dictionary format
annotation_rev = {}
for i in cluster_list:
    for k in annotation:
        if int(i) in annotation[k]:
            annotation_rev[i] = k

# check dictionary
annotation_rev
```

```
[22]: {'5': 'MEP',
       '2': 'Monocytes',
       '12': 'Erythroids',
       '13': 'Granulocytes',
       '0': 'late_GMP',
       '10': 'Erythroids',
       '3': 'Erythroids',
       '18': 'Erythroids',
       '11': 'GMP',
       '7': 'Granulocytes',
       '8': 'Erythroids',
       '22': 'Megakaryocytes',
       '16': 'Erythroids',
       '1': 'GMP',
       '17': 'Megakaryocytes',
       '4': 'Granulocytes',
       '19': 'Erythroids',
       '9': 'Erythroids',
       '6': 'Monocytes',
       '20': 'Lymphoid',
       '14': 'Erythroids',
       '15': 'Erythroids',
       '21': 'DC'}
```

```
[23]: adata.obs["cell_type"] = [annotation_rev[i] for i in adata.obs.louvain]
```

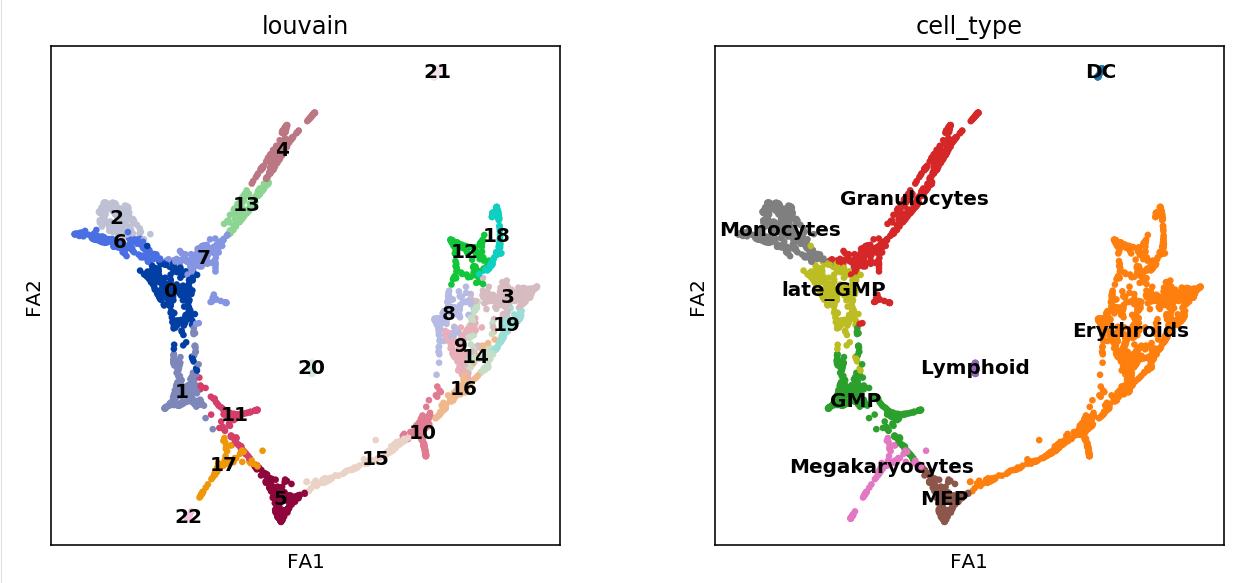
```
[24]: # check results
sc.pl.draw_graph(adata, color=['cell_type', 'paul15_clusters'],
                 legend_loc='on data')
... storing 'cell_type' as categorical
```



## 9.2. Make annotation (2)

We'll make another annotation manually for each Louvain clusters.

```
[25]: sc.pl.draw_graph(adata, color=['louvain', 'cell_type'],
                     legend_loc='on data')
```

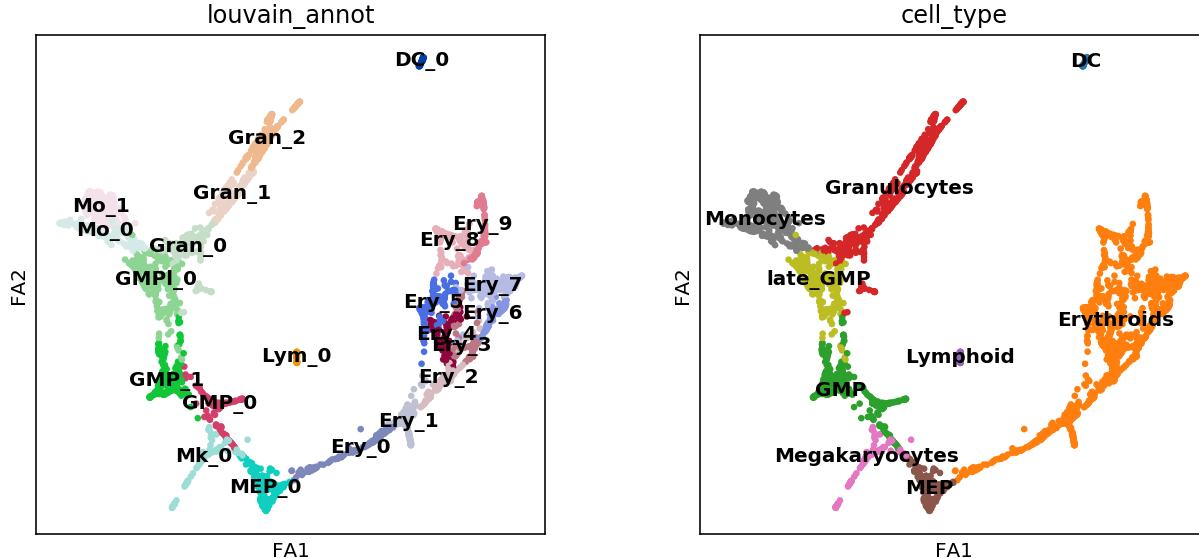


!! Please change the dictionary below depending on the clustering results. The results may change depending on the execution environment.

```
[26]: annotation_2 = { '5': 'MEP_0',
                     '15': 'Ery_0',
                     '10': 'Ery_1',
                     '16': 'Ery_2',
                     '14': 'Ery_3',
                     '9': 'Ery_4',
                     '8': 'Ery_5',
                     '19': 'Ery_6',
                     '3': 'Ery_7',
                     '12': 'Ery_8',
                     '18': 'Ery_9',
                     '17': 'Mk_0',
                     '22': 'Mk_0',
                     '11': 'GMP_0',
                     '1': 'GMP_1',
                     '0': 'GMP1_0',
                     '7': 'Gran_0',
                     '13': 'Gran_1',
                     '4': 'Gran_2',
                     '6': 'Mo_0',
                     '2': 'Mo_1',
                     '21': 'DC_0',
                     '20': 'Lym_0'}
```

```
[27]: adata.obs["louvain_annot"] = [annotation_2[i] for i in adata.obs.louvain]
```

```
[28]: # Check result
sc.pl.draw_graph(adata, color=['louvain_annot', 'cell_type'],
                 legend_loc='on data')
... storing 'louvain_annot' as categorical
```



We've done several scRNA-preprocessing steps; filtering, normalization, clustering, and dimensional reduction. In the next step, we'll do the GRN inference, network analysis, and in silico simulation based on this information.

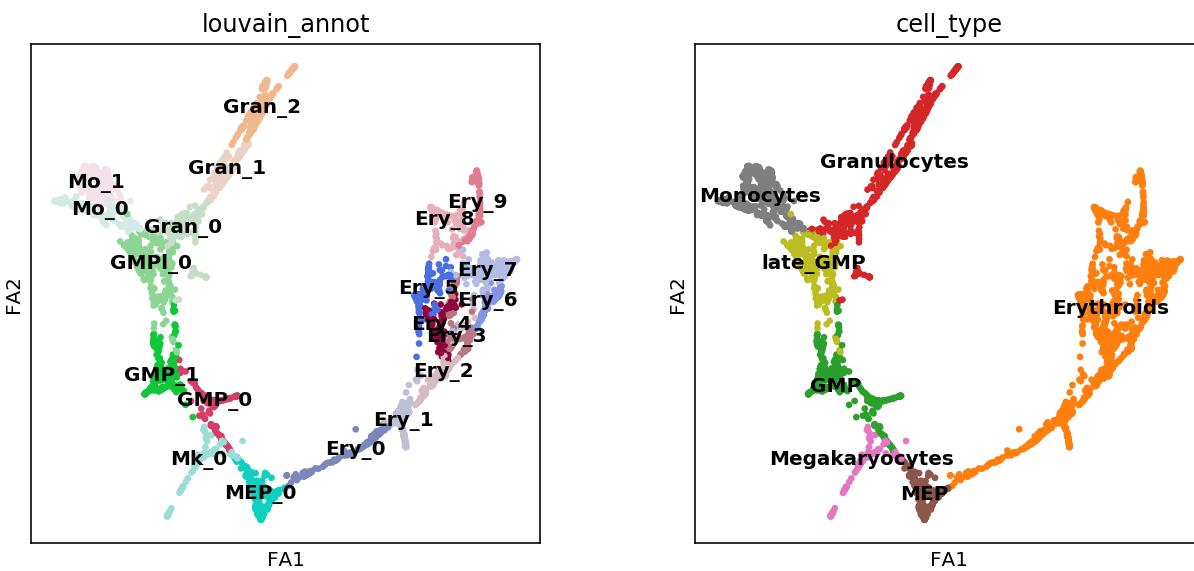
## 10. (Option) Subset cells

In this tutorial, we are using scRNA-seq data of hematopoiesis. In the latter part, we will focus on the cell fate decision in the myeloid lineage. So we will remove non-myeloid cell cluster; DC and Lymphoid cell cluster.

```
[29]: adata.obs.cell_type.unique()
[29]: [MEP, Monocytes, Erythroids, Granulocytes, late_GMP, GMP, Megakaryocytes, Lymphoid, DC]
Categories (9, object): [MEP, Monocytes, Erythroids, Granulocytes, ..., GMP, Megakaryocytes, Lymphoid, DC]
```

```
[30]: cell_of_interest = adata.obs.index[~adata.obs.cell_type.isin(["Lymphoid", "DC"])]
adata = adata[cell_of_interest, :]
```

```
[31]: # check result
sc.pl.draw_graph(adata, color=['louvain_annot', 'cell_type'],
                 legend_loc='on data')
```



## 11. Save data

```
[32]: adata.write_h5ad("data/Paul_et al_15.h5ad")
```

## B. scRNA-seq data preprocessing with Seurat

R notebook ... comming in the future update.

---

**Note:** If you use Seurat for preprocessing, you need to convert the scRNA-seq data (Seurat object) into anndata to analyze the data with celloracle. celloracle has a python API and command-line API to convert a Seurat object into an anndata. Please go to the documentation of celloracle's API documentation for more information.

## 1.2.4 Network analysis

celloracle imports the scRNA-seq dataset and TF binding information to find active regulatory connections for all genes, generating sample-specific GRNs.

The inferred GRN is analyzed with several network algorithms to get various network scores. The network score is useful to identify key regulatory genes.

Celloracle reconstructs a GRN for each cluster, enabling us to compare GRNs to each other. It is also possible to analyze how the GRN changes over differentiation. The dynamics of the GRN structure can provide us insight into the context-dependent regulatory mechanisms.

The jupyter notebook files and data used in this tutorial are available [here](#).

Python notebook

### 0. Import libraries

```
[1]: # 0. Import

import os
import sys

import matplotlib.pyplot as plt
import numpy as np
import pandas as pd
import scanpy as sc
import seaborn as sns
```

```
[2]: import celloracle as co
co.__version__
```

```
[2]: '0.5.0'
```

```
[7]: # visualization settings
%config InlineBackend.figure_format = 'retina'
%matplotlib inline

plt.rcParams['figure.figsize'] = [6, 4.5]
plt.rcParams["savefig.dpi"] = 300
```

### 0.1. Check installation

Celloracle uses some R libraries in network analysis. Please make sure that all dependent R libraries are installed on your computer. You can test the installation with the following command.

```
[4]: co.network_analysis.test_R_libraries_installation()

R path: /usr/bin/R
checking R library installation: igraph -> OK
checking R library installation: linkcomm -> OK
checking R library installation: rnetcarto -> OK
```

## 0.2. Make a folder to save graph

```
[5]: save_folder = "figures"
os.makedirs(save_folder, exist_ok=True)
```

## 1. Load data

### 1.1. Load processed gene expression data (anndata)

Please refer to the previous notebook in the tutorial for an example of how to process scRNA-seq data.

```
[6]: # Load data. !!Replace the data path below when you use another data.
adata = sc.read_h5ad("../03_scRNA-seq_data_preprocessing/data/Paul_et al_15.h5ad")

/home/k/anaconda3/envs/pandas1/lib/python3.6/site-packages/anndata/compat/__init__.py:
  ↪161: FutureWarning: Moving element from .uns['neighbors']['distances'] to .obsp[
  ↪'distances'].

This is where adjacency matrices should go now.
  FutureWarning,
/home/k/anaconda3/envs/pandas1/lib/python3.6/site-packages/anndata/compat/__init__.py:
  ↪161: FutureWarning: Moving element from .uns['neighbors']['connectivities'] to .
  ↪obsp['connectivities'].

This is where adjacency matrices should go now.
  FutureWarning,
```

### 1.2. Load TF data.

For the GRN inference, celloracle needs TF information, which contains lists of the regulatory candidate genes. There are several ways to make such TF information. We can generate TF information from scATAC-seq data or bulk ATAC-seq data. Please refer to the first step of the tutorial for the details of this process.

If you do not have your scATAC-seq data, you can use some built-in data in celloracle. The built-in TFinfo wqs made using various tissue/cell-types from the mouse ATAC-seq atlas dataset (<http://atlas.gs.washington.edu/mouse-atac/>).

You can load and use the data with the following command.

```
[11]: # Load TF info which was made from mouse cell atlas dataset.
TFinfo_df = co.data.load_TFinfo_df_mm9_mouse_atac_atlas()

# Check data
TFinfo_df.head()
```

	peak_id	gene_short_name	9430076c15rik	Ac002126.6	\						
0	chr10_100050979_100052296	4930430F08Rik		0.0	0.0						
1	chr10_101006922_101007748	SNORA17		0.0	0.0						
2	chr10_101144061_101145000	Mgat4c		0.0	0.0						
3	chr10_10148873_10149183	9130014G24Rik		0.0	0.0						
4	chr10_10149425_10149815	9130014G24Rik		0.0	0.0						
	Ac012531.1	Ac226150.2	Afp	Ahr	Ahrr	Aire	...	Znf784	Znf8	Znf816	\
0	1.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	0.0

(continues on next page)

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2	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0
<hr/>										
Znf85	Zscan10	Zscan16	Zscan22	Zscan26	Zscan31	Zscan4				
0	0.0	0.0	0.0	0.0	0.0	0.0				
1	0.0	0.0	0.0	0.0	0.0	1.0	0.0			
2	0.0	0.0	0.0	0.0	0.0	0.0	1.0			
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		

[5 rows x 1095 columns]

## 2. Initiate Oracle object

Celloracle has a custom called Oracle. We can use Oracle for the data preprocessing and GRN inference steps. The Oracle object stores all of necessary information and does the calculations with its internal functions. We instantiate an Oracle object, then input the gene expression data (anndata) and a TFinfo into the Oracle object.

```
[7]: # Instantiate Oracle object
oracle = co.Oracle()
```

### 2.1. load gene expression data into oracle object.

When you load a scRNA-seq data, please enter the name of clustering data and dimensional reduction data. The clustering data should be stored in the attribute of “obs” in the anndata. Dimensional reduction data suppose to be stored in the attribute of “obsm” in the anndata. You can check these data by the following command.

If you are not familiar with anndata, please look at the documentation of annata (<https://anndata.readthedocs.io/en/stable/>) or Scanpy (<https://scanpy.readthedocs.io/en/stable/>).

For the celloracle analysis, the anndata shoud include (1) gene expression count, (2) clustering information, (3) trajectory (dimensional reduction embeddings) data. Please refer to another notebook for more information on anndata preprocessing.

```
[8]: # show data name in anndata
print("metadata columns : ", list(adata.obs.columns))
print("dimensional reduction: ", list(adata.obsm.keys()))
metadata columns : ['paul15_clusters', 'n_counts_all', 'n_counts', 'louvain', 'cell_type', 'louvain_annot']
dimensional reduction: ['X_diffmap', 'X_draw_graph_fa', 'X_pca']
```

```
[9]: # In this notebook, we use raw mRNA count as an input of Oracle object.
adata.X = adata.raw.X.copy()

# Instantiate Oracle object.
oracle.import_anndata_as_raw_count(adata=adata,
                                    cluster_column_name="louvain_annot",
                                    embedding_name="X_draw_graph_fa")
```

## 2.2. Load TFinfo into oracle object

```
[13]: # You can load TF info dataframe with the following code.
oracle.import_TF_data(TF_info_matrix=TFinfo_df)

# Alternatively, if you saved the information as a dictionary, you can use the code below.
# oracle.import_TF_data(TFdict=TFinfo_dictionary)
```

## 2.3. (Optional) Add TF info manually

While we mainly use TF info data made from scATAC-seq data, we can also add additional information about the TF-target gene pair manually.

For example, if there is a study or database that includes specific TF-target pairs, you can use such information in the following way.

### 2.3.1. Make TF info dictionary manually

Here, we will introduce how to add TF binding information.

We will start with TF binding data from supplemental table 4 in (<http://doi.org/10.1016/j.cell.2015.11.013>).

In order to import TF data into the Oracle object, we need to convert them into a python dictionary. The dictionary keys will be the target genes, and the values will be the regulatory candidate TFs.

```
[50]: # We have TF and its target gene information. This is from a supplemental Fig of Paul et. al, (2015).
Paul_15_data = pd.read_csv("TF_data_in_Paul15.csv")
Paul_15_data
```

	TF	Target_genes
0	Cebpa	Abcb1b, Acot1, C3, Cnpy3, Dhrs7, Dtx4, Edem2, ...
1	Irf8	Abcd1, Aif1, BC017643, Cbl, Ccdc109b, Ccl6, d6...
2	Irf8	1100001G20Rik, 4732418C07Rik, 9230105E10Rik, A...
3	Klf1	2010011I20Rik, 5730469M10Rik, Acsl6, Add2, Ank...
4	Sfp1l	0910001L09Rik, 2310014H01Rik, 4632428N05Rik, A...

```
[51]: # Make dictionary: dictionary Key is TF, dictionary Value is list of target genes
TF_to_TG_dictionary = {}

for TF, TGs in zip(Paul_15_data.TF, Paul_15_data.Target_genes):
    # convert target gene to list
    TG_list = TGs.replace(" ", "").split(",")
    # store target gene list in a dictionary
    TF_to_TG_dictionary[TF] = TG_list

# We have to make a dictionary, in which a Key is Target gene and value is TF.
# We invert the dictionary above using a utility function in celloracle.
TG_to_TF_dictionary = co.utility.inverse_dictionary(TF_to_TG_dictionary)

HBox(children=(FloatProgress(value=0.0, max=178.0), HTML(value='')))
```

### 2.3.2. Add TF information dictionary into the oracle object

```
[53]: # Add TF information
oracle.addTFinfo_dictionary(TG_to_TF_dictionary)
```

## 3. Knn imputation

Celloracle uses almost the same strategy as velocyto for visualizing cell transitions. This process requires KNN imputation in advance.

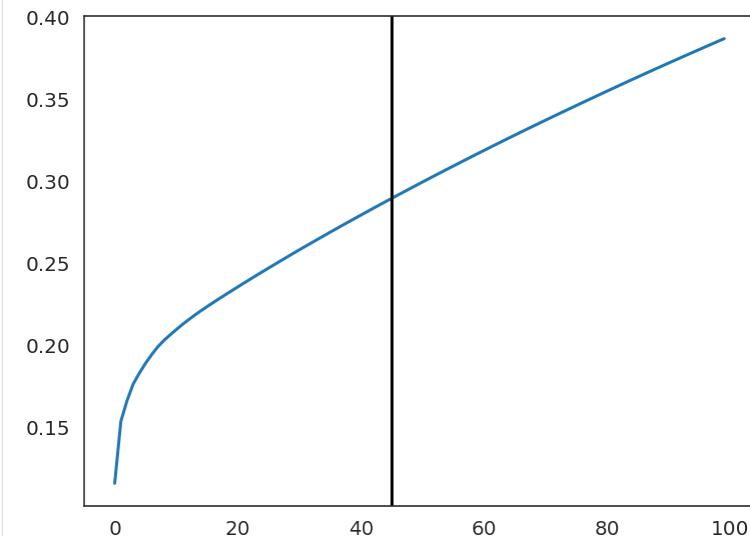
For the KNN imputation, we need PCA and PC selection first.

### 3.1. PCA

```
[60]: # Perform PCA
oracle.perform_PCA()

# Select important PCs
plt.plot(np.cumsum(oracle.pca.explained_variance_ratio_) [:100])
n_comps = np.where(np.diff(np.diff(np.cumsum(oracle.pca.explained_variance_ratio_)) > 0.
                           ↪ 0.002)) [0] [0]
plt.axvline(n_comps, c="k")
print(n_comps)
n_comps = min(n_comps, 50)
```

45



### 3.2. KNN imputation

Estimate the optimal number of nearest neighbors for KNN imputation.

```
[63]: n_cell = oracle.adata.shape[0]
print(f"cell number is :{n_cell}")

cell number is :2671
```

```
[64]: k = int(0.025*n_cell)
print(f"Auto-selected k is :{k}")

Auto-selected k is :66
```

```
[65]: oracle.knn_imputation(n_pca_dims=n_comps, k=k, balanced=True, b_sight=k*8,
                           b_maxl=k*4, n_jobs=4)
```

### 4. Save and Load.

Celloracle has some custom-classes: Links, Oracle and TFinfo. You can save such an object using “to\_hdf5”.

Please use “load\_hdf5” function to load the file.

```
[66]: # Save oracle object.
oracle.to_hdf5("Paul_15_data.celloracle.oracle")
```

```
[19]: # Load file.
#oracle = co.load_hdf5("Paul_15_data.celloracle.oracle")
```

### 5. GRN calculation

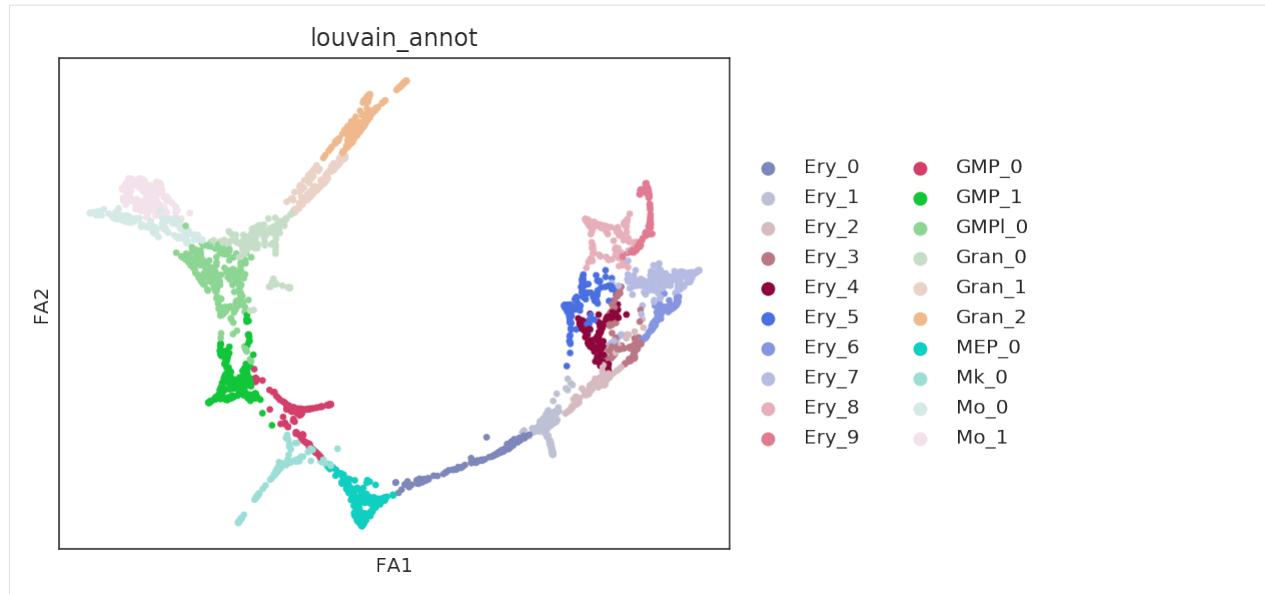
The next step is constructing a cluster-specific GRN for all clusters.

You can calculate GRNs with the “get\_links” function, and the function returns GRNs as a Links object. The Links object stores inferred GRNs and the corresponding metadata. You can do network analysis with the Links object.

The GRN will be calculated for each cluster/sub-group. In the example below, we construct GRN for each unit of the “louvain\_annot” clustering.

The GRNs can be calculated at any arbitrary unit as long as the clustering information is stored in anndata.

```
[67]: # check data
sc.pl.draw_graph(oracle.adata, color="louvain_annot")
```



## 5.1. Get GRNs

```
[ ]: %%time
# Calculate GRN for each population in "louvain_annot" clustering unit.
# This step may take long time.
links = oracle.get_links(cluster_name_for_GRN_unit="louvain_annot", alpha=10,
                         verbose_level=10, test_mode=False)
```

## 5.2. (Optional) Export GRNs

Although celloracle has many functions for network analysis, you can analyze GRNs by hand if you choose. The raw GRN data is stored in the attribute of “links\_dict”.

For example, you can get the GRN for the “Ery\_0” cluster with the following commands.

```
[72]: links.links_dict.keys()
[72]: dict_keys(['Ery_0', 'Ery_1', 'Ery_2', 'Ery_3', 'Ery_4', 'Ery_5', 'Ery_6', 'Ery_7',
   ↪'Ery_8', 'Ery_9', 'GMP_0', 'GMP_1', 'GMPI_0', 'Gran_0', 'Gran_1', 'Gran_2', 'MEP_0',
   ↪ 'Mk_0', 'Mo_0', 'Mo_1'])
```

```
[73]: links.links_dict["Ery_0"]
[73]:      source      target  coef_mean  coef_abs      p      -logp
0       Stat3  0610007L01Rik  -0.010275  0.010275  3.476931e-07  6.458804
1       Gata1  0610007L01Rik  -0.000380  0.000380  7.598357e-01  0.119280
2       Zbtb1  0610007L01Rik   0.004452  0.004452  1.018526e-03  2.992028
3       Rara  0610007L01Rik  -0.000669  0.000669  7.065405e-01  0.150863
4        Myc  0610007L01Rik  -0.010705  0.010705  1.696471e-05  4.770454
...
...      ...      ...      ...      ...      ...      ...      ...
(continues on next page)
```

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74420	Smarcc2	Zyx	-0.003475	0.003475	2.754236e-02	1.559999
74421	Nfe2	Zyx	0.031430	0.031430	1.461503e-11	10.835200
74422	Zbtb4	Zyx	0.001684	0.001684	1.915555e-01	0.717705
74423	Smarcc1	Zyx	0.011356	0.011356	1.843519e-04	3.734352
74424	Nfkbl1	Zyx	0.010803	0.010803	1.805959e-06	5.743292

[74425 rows x 6 columns]

You can export the file as follows.

```
[ ]: # Set cluster name
cluster = "Ery_0"

# Save as csv
links.links_dict[cluster].to_csv(f"raw_GRN_for_{cluster}.csv")
```

### 5.3. (Optional) Change order

The links object has a color information in an attribute, “palette”. This information is used for the visualization

The sample will be visualized in that order. Here we can change the order.

```
[75]: # Show the contents of palette
links.palette
```

```
[75]: palette
MEP_0      #0FCFC0
Mk_0       #9CDED6
Ery_0      #7D87B9
Ery_1      #BEC1D4
Ery_2      #D6BCC0
Ery_3      #BB7784
Ery_4      #8E063B
Ery_5      #4A6FE3
Ery_6      #8595E1
Ery_7      #B5BBE3
Ery_8      #E6AFB9
Ery_9      #E07B91
GMP_0      #D33F6A
GMP_1      #11C638
GMP1_0     #8DD593
Mo_0       #D5EAE7
Mo_1       #F3E1EB
Gran_0     #C6DEC7
Gran_1     #EAD3C6
Gran_2     #F0B98D
```

```
[76]: # Change the order of palette
order = ['MEP_0', 'Mk_0', 'Ery_0', 'Ery_1', 'Ery_2', 'Ery_3', 'Ery_4', 'Ery_5',
         'Ery_6', 'Ery_7', 'Ery_8', 'Ery_9', 'GMP_0', 'GMP_1',
         'GMP1_0', 'Mo_0', 'Mo_1', 'Gran_0', 'Gran_1', 'Gran_2']
links.palette = links.palette.loc[order]
links.palette
```

```
[76]: palette
MEP_0      #0FCFC0
```

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```
Mk_0      #9CDED6
Ery_0    #7D87B9
Ery_1    #BEC1D4
Ery_2    #D6BCC0
Ery_3    #BB7784
Ery_4    #8E063B
Ery_5    #4A6FE3
Ery_6    #8595E1
Ery_7    #B5BBE3
Ery_8    #E6AFB9
Ery_9    #E07B91
GMP_0    #D33F6A
GMP_1    #11C638
GMP1_0   #8DD593
Mo_0     #D5EAE7
Mo_1     #F3E1EB
Gran_0   #C6DEC7
Gran_1   #EAD3C6
Gran_2   #F0B98D
```

## 6. Network preprocessing

### 6.1. Filter network edges

Celloracle utilizes bagging ridge or Bayesian ridge regression to infer gene regulatory networks. These methods provide a network edge strength as a distribution rather than a point value. We can use the distribution to know the certainness of the connection.

We filter the network edges as follows.

- (1) Remove uncertain network edges based on the p-value.
- (2) Remove weak network edge. In this tutorial, we pick up the top 2000 edges in terms of network strength.

The raw network data is stored as an attribute, “links\_dict,” while filtered network data is stored in “filtered\_links.” Thus the filtering function keeps raw network information rather than overwriting the data. You can come back to the filtering process to filter the data with different parameters if you want.

```
[78]: links.filter_links(p=0.001, weight="coef_abs", thread_number=2000)
```

### 6.2. Degree distribution

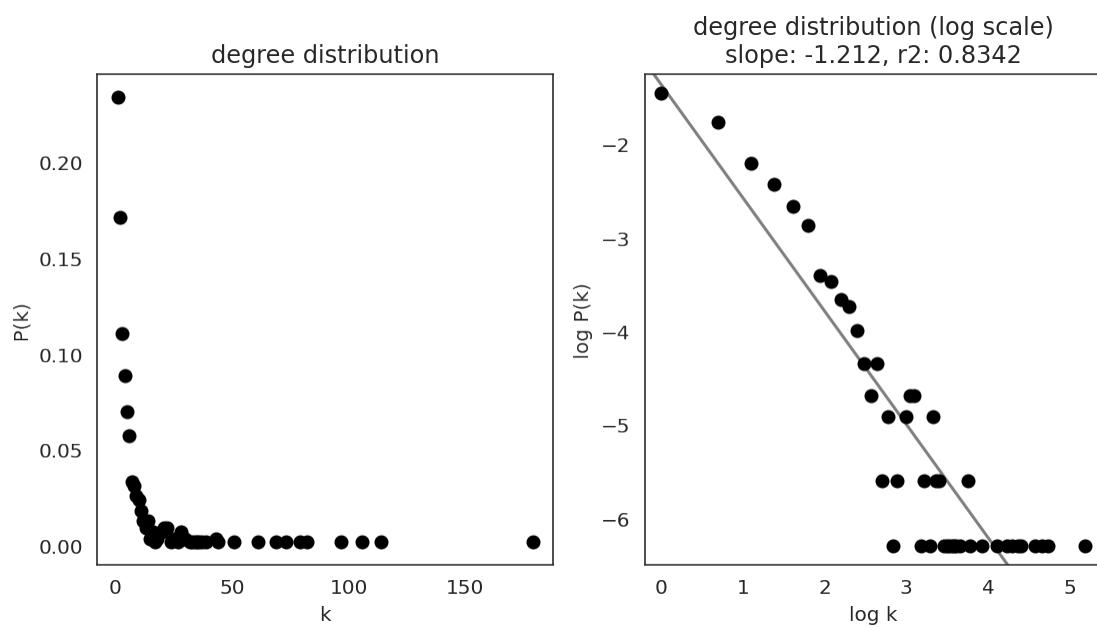
In the first step, we examine the network degree distribution. Network degree, which is the number of edges for each node, is one of the important metrics used to investigate the network structure ([https://en.wikipedia.org/wiki/Degree\\_distribution](https://en.wikipedia.org/wiki/Degree_distribution)).

Please keep in mind that the degree distribution may change depending on the filtering threshold.

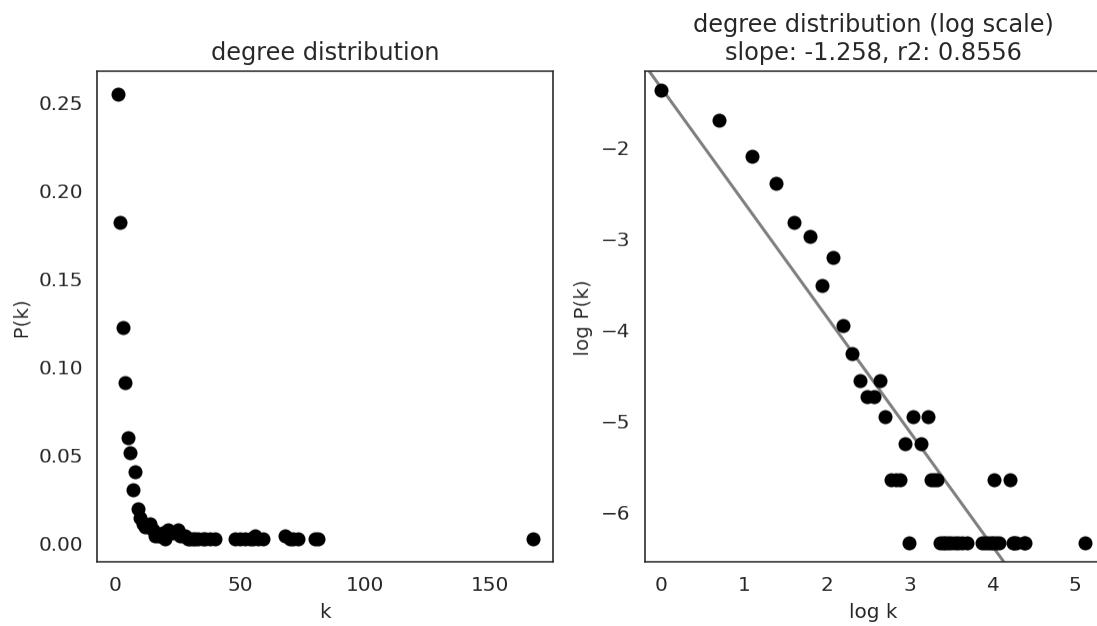
```
[9]: plt.rcParams["figure.figsize"] = [9, 4.5]
```

```
[10]: links.plot_degree_distributions(plot_model=True,
                                     #save=f"{save_folder}/degree_
                                     ↪distribution/",
                                     )
```

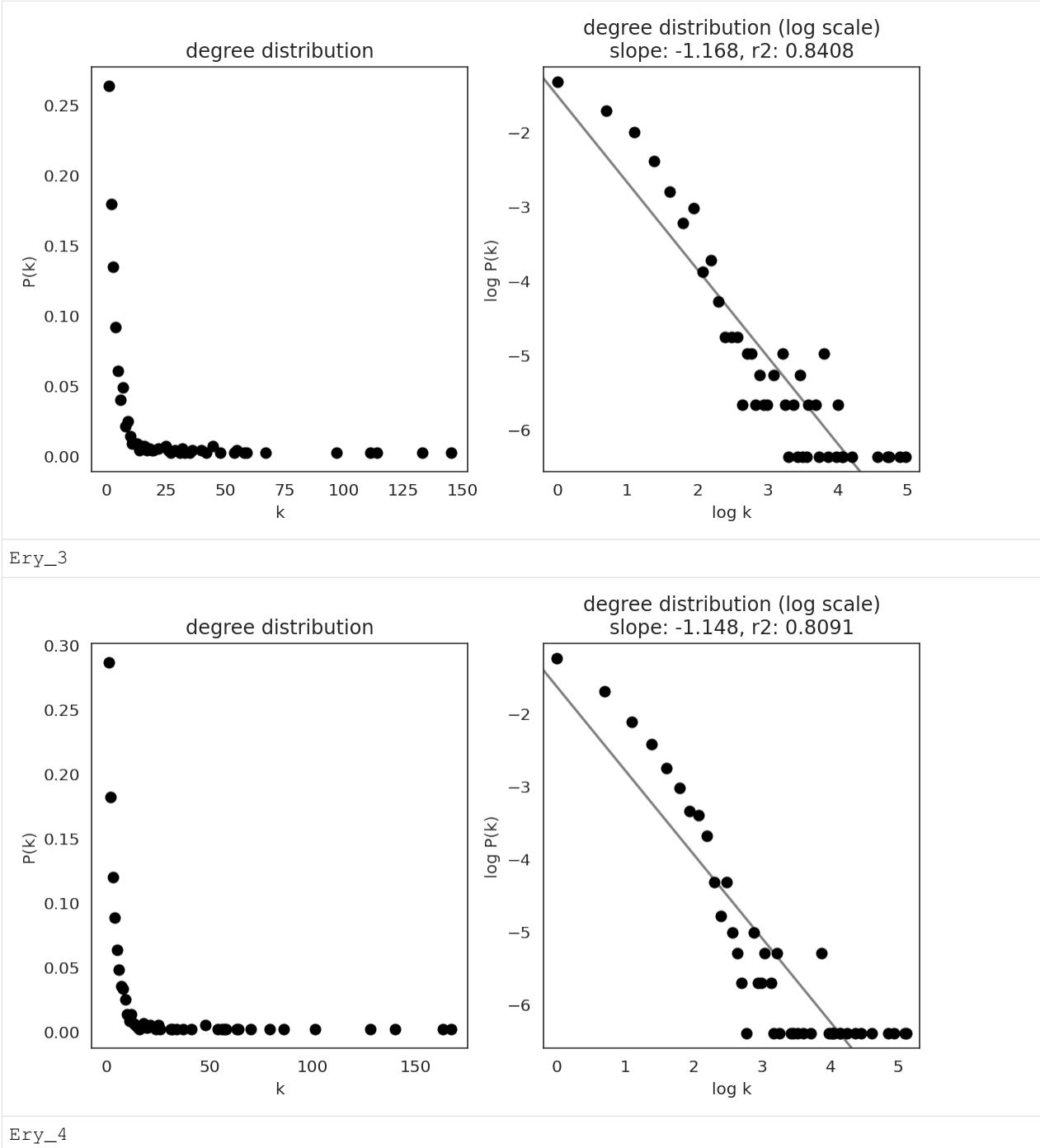
Ery\_0

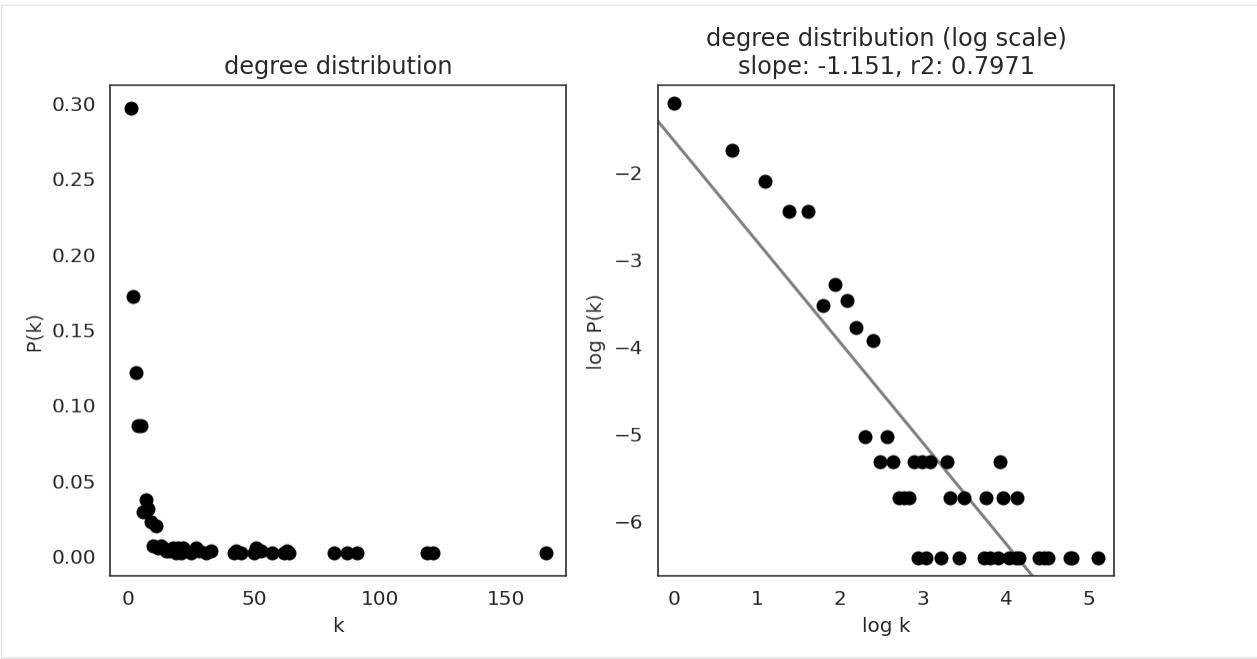


Ery\_1

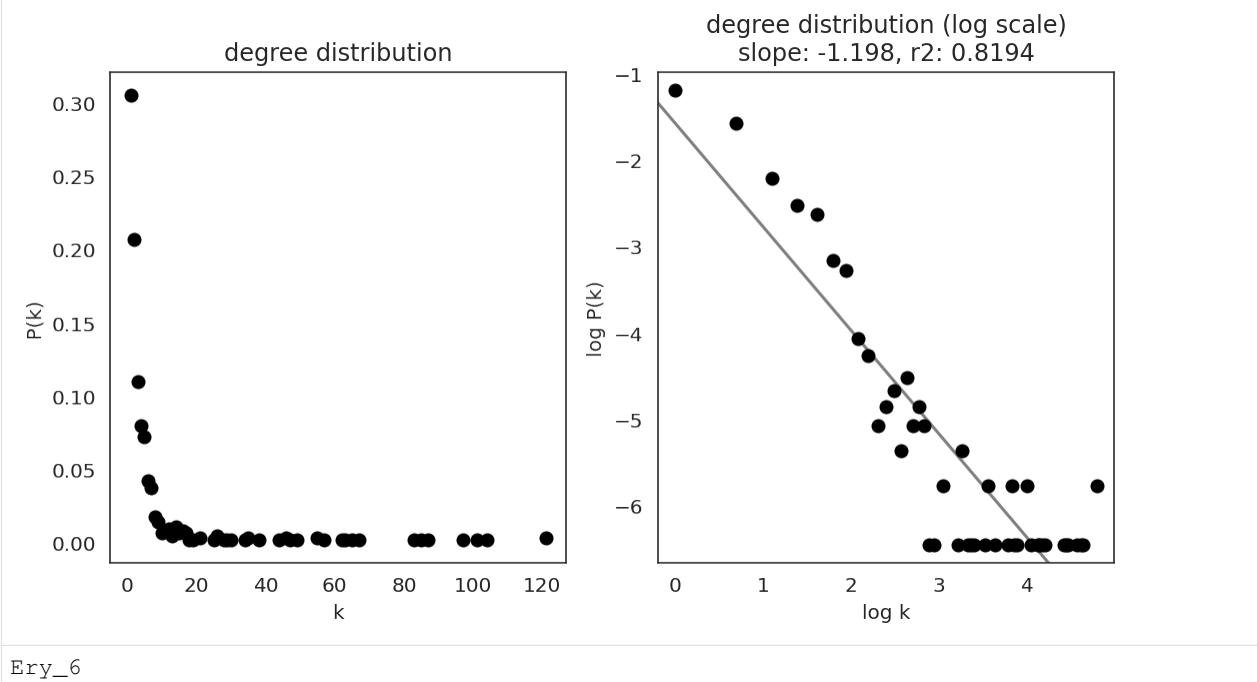


Ery\_2

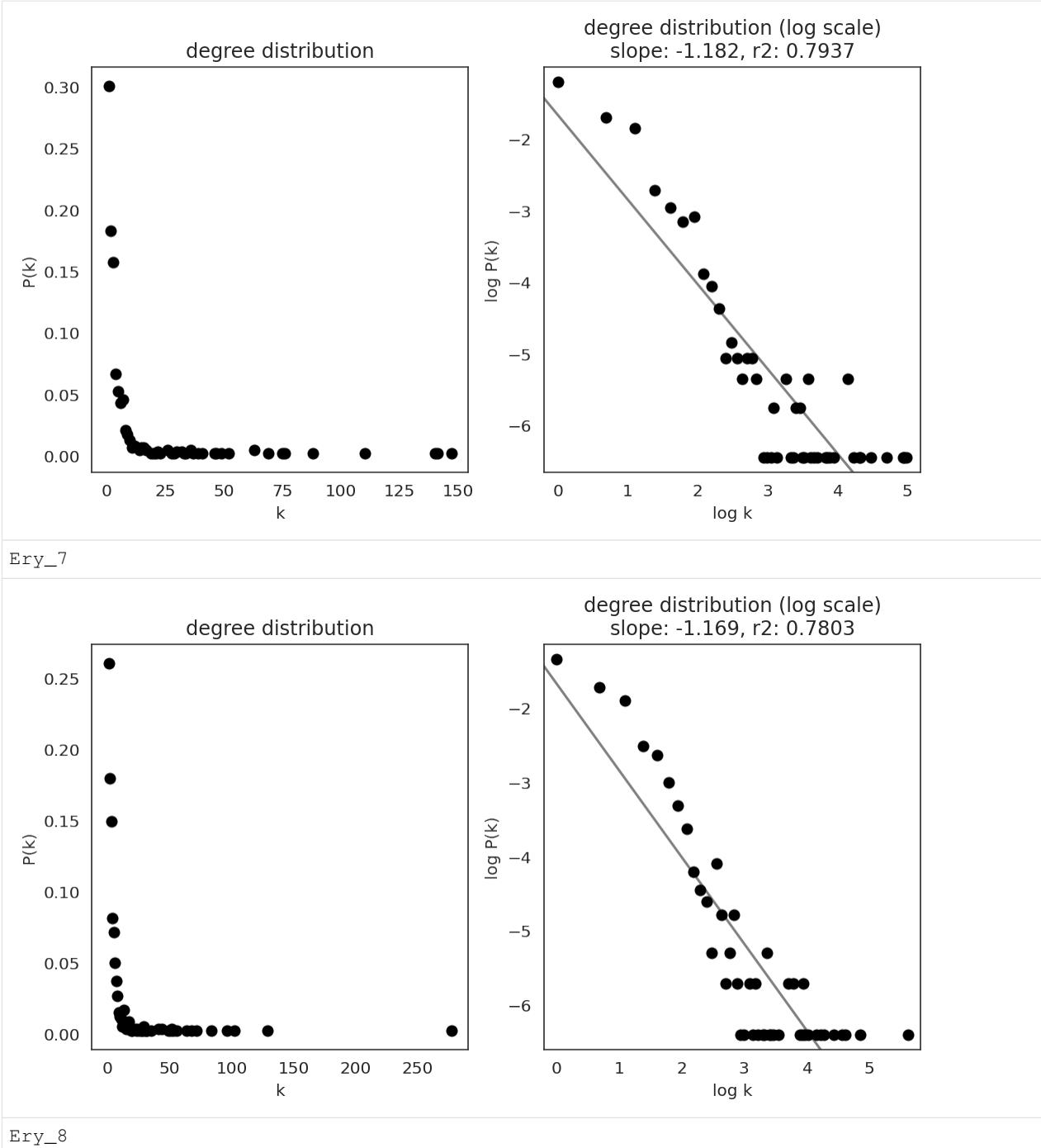


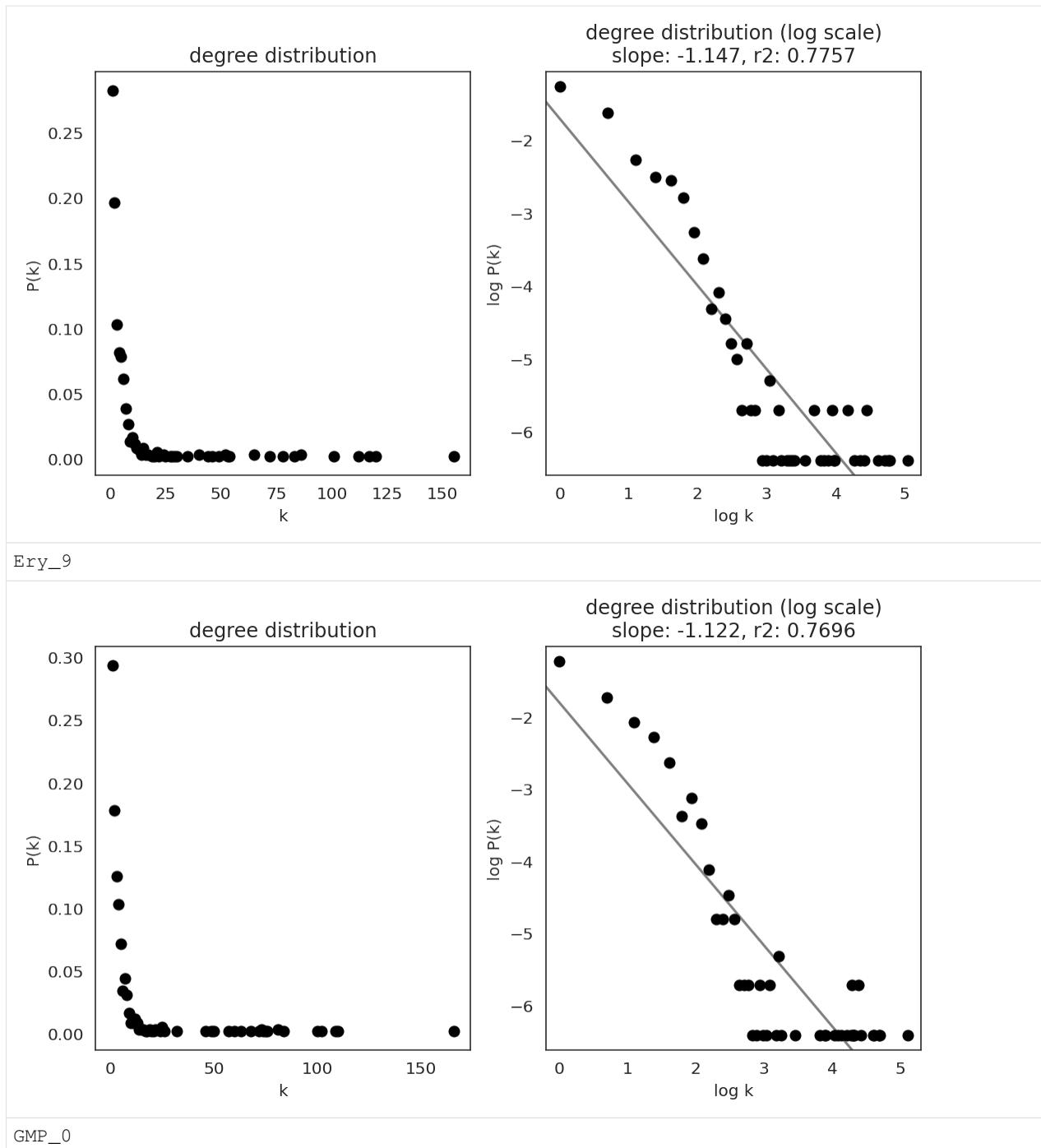


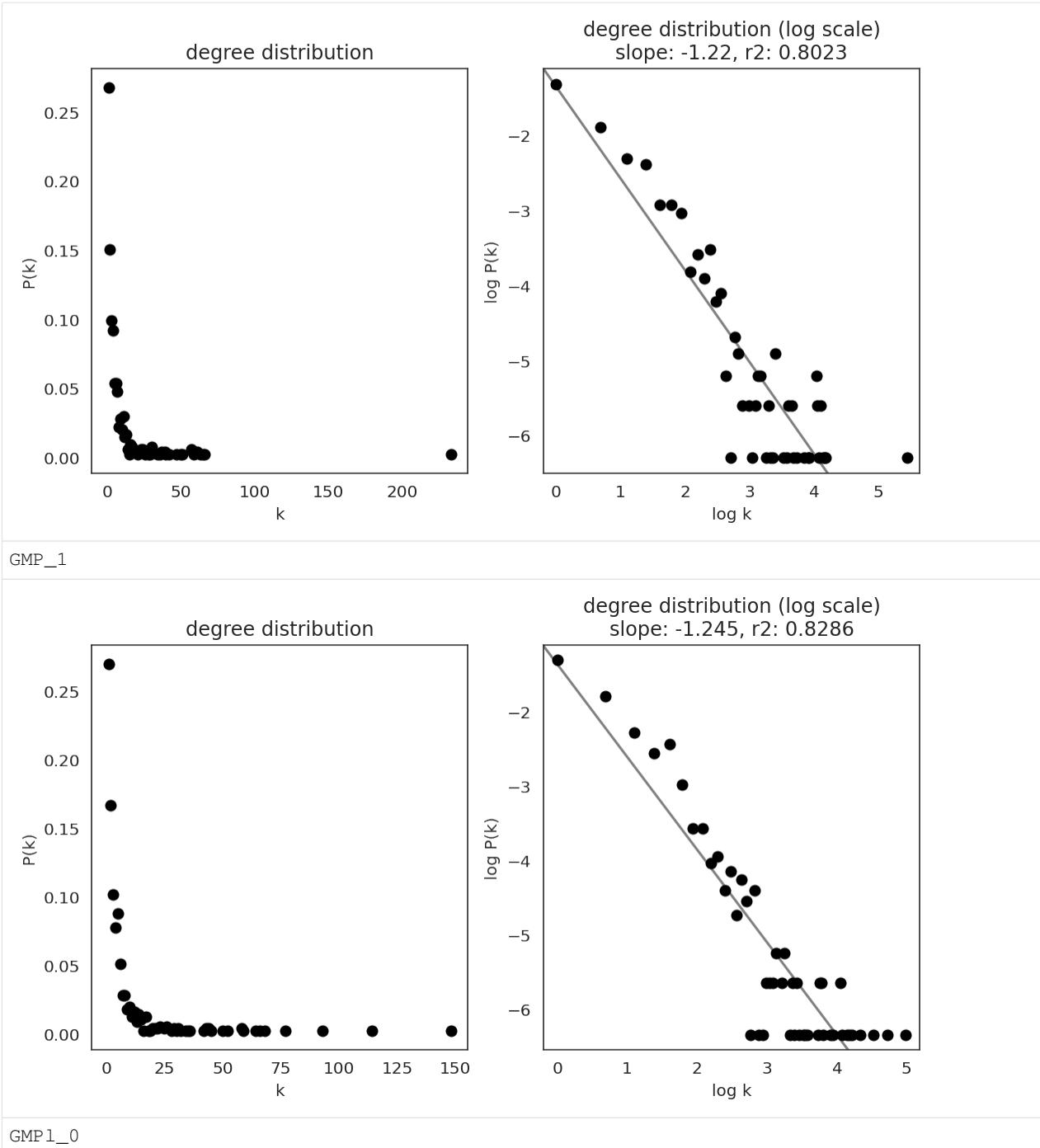
Ery\_5

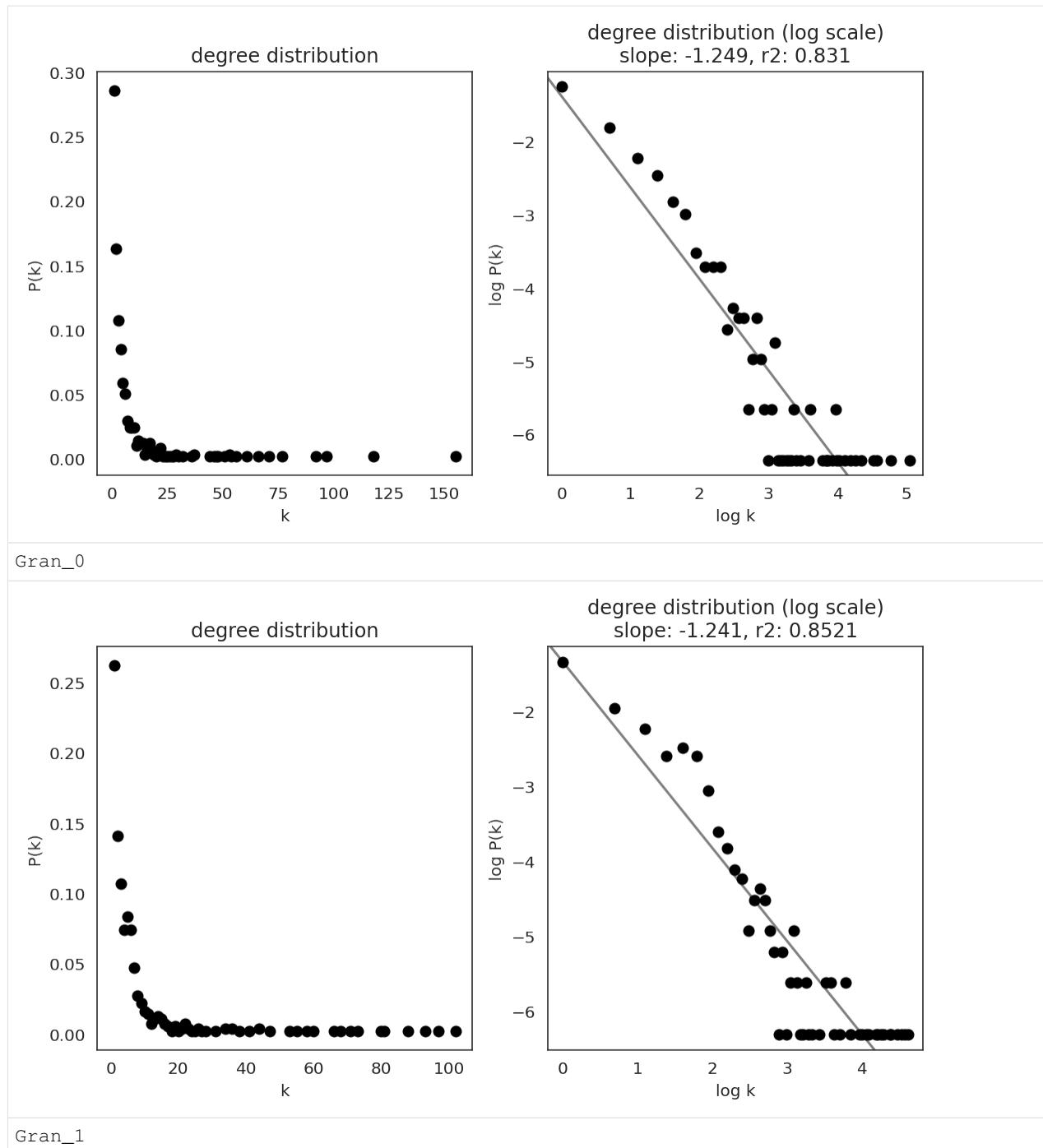


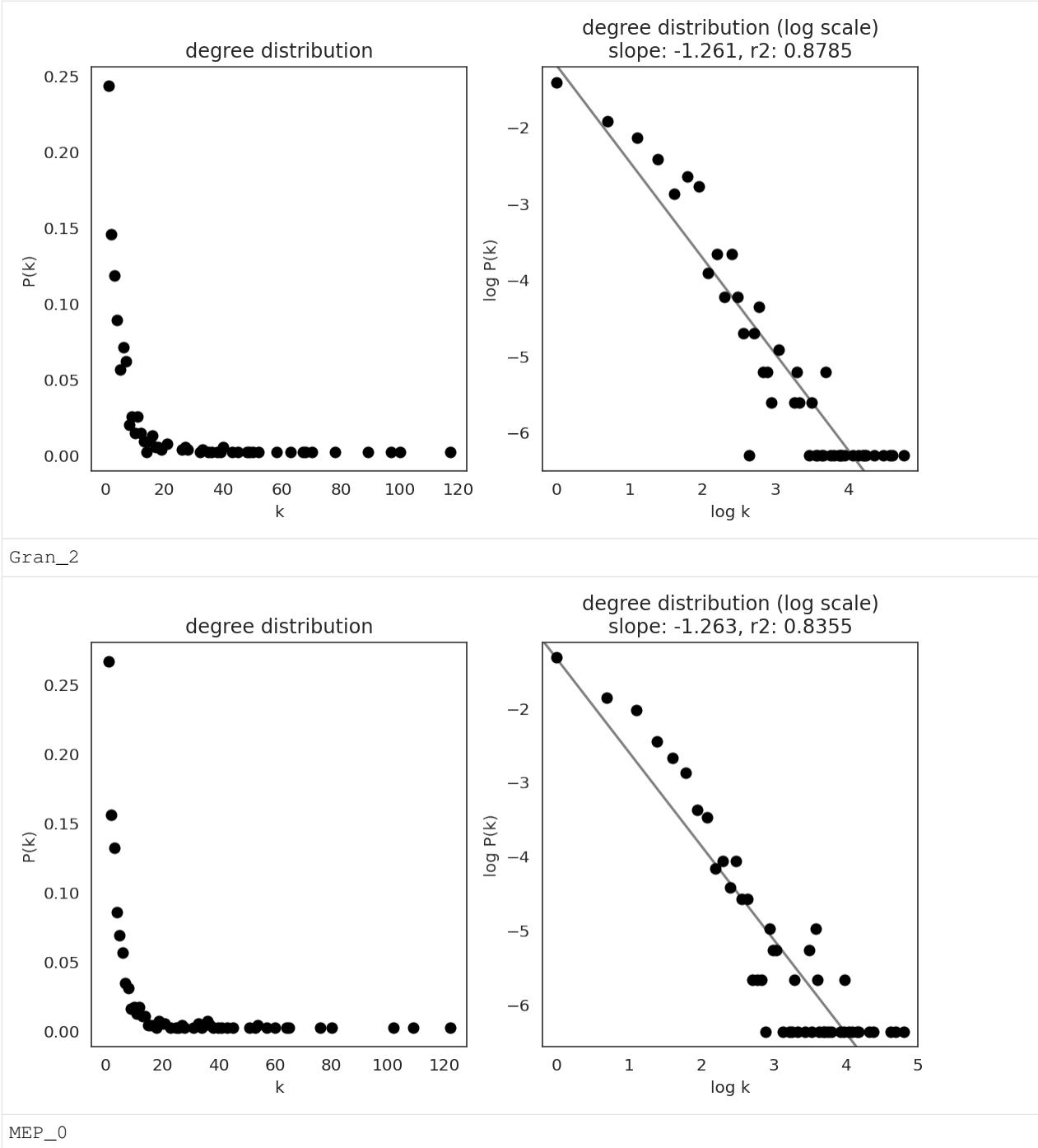
Ery\_6

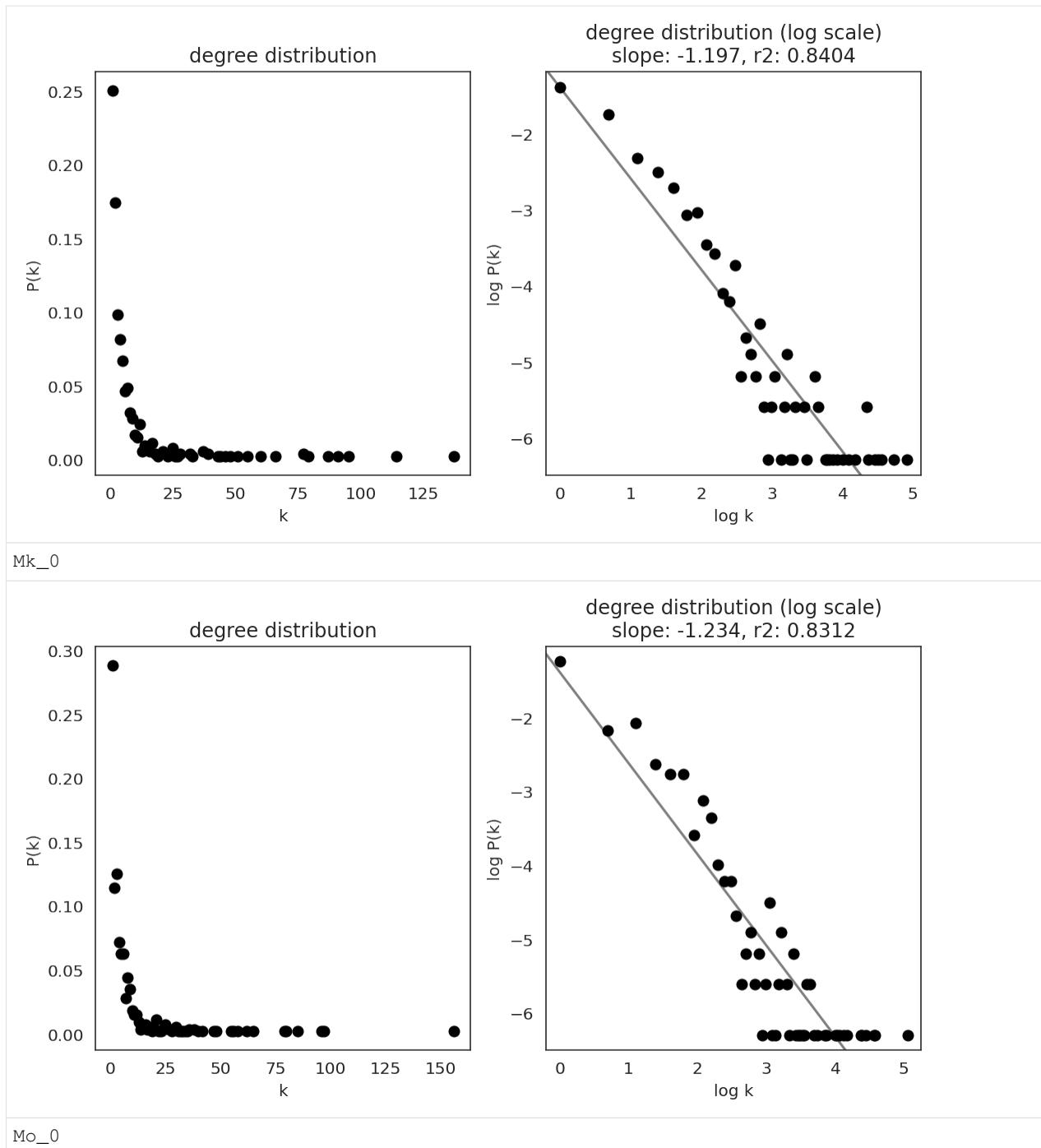


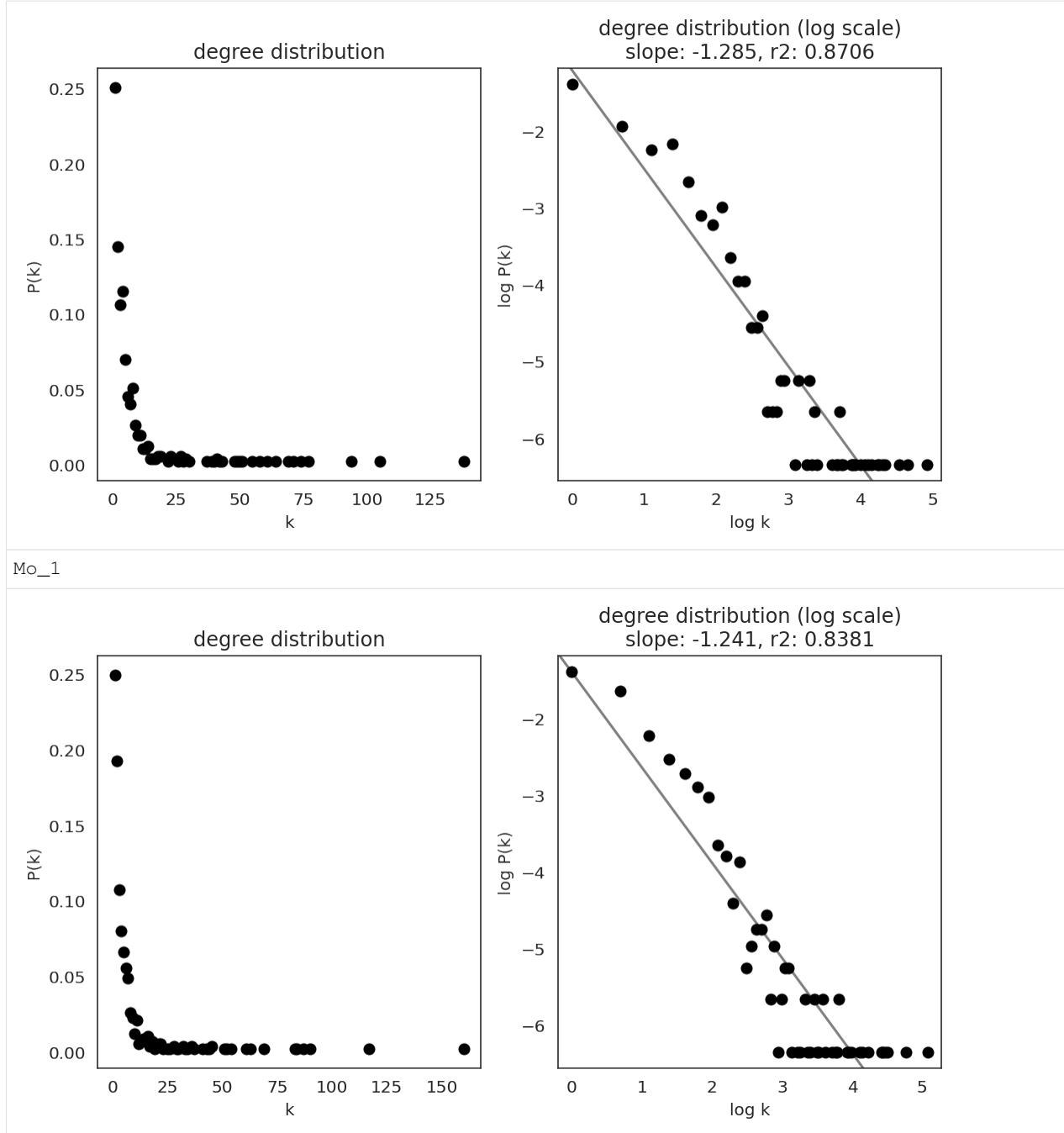












```
[28]: plt.rcParams["figure.figsize"] = [6, 4.5]
```

### 5.3. Calculate netowrk score

Next, we calculate several network score using some R libraries. Please make sure that R libraries are installed in your PC before running the command below.

```
[25]: # Calculate network scores. It takes several minutes.
links.get_score()

processing... batch 1/3
Ery_0: finished.
Ery_1: finished.
Ery_2: finished.
Ery_3: finished.
Ery_4: finished.
Ery_5: finished.
Ery_6: finished.
Ery_7: finished.
processing... batch 2/3
Ery_8: finished.
Ery_9: finished.
GMP_0: finished.
GMP_1: finished.
GMP1_0: finished.
Gran_0: finished.
Gran_1: finished.
Gran_2: finished.
processing... batch 3/3
MEP_0: finished.
Mk_0: finished.
Mo_0: finished.
Mo_1: finished.
```

The score is stored as a attribute called “merged\_score”, and the score will also be saved in a folder in your computer.

```
[82]: links.merged_score.head()

[82]:      degree_all  degree_in  degree_out  clustering_coefficient \
Stat3          82          0         82          0.021981
Mycn          30          0         30          0.011494
E2f4         181          2        179          0.009822
Zbtb1          22          0         22          0.000000
Ybx1          69          9         60          0.028133

      clustering_coefficient_weighted  degree_centrality_all \
Stat3                  0.022055          0.151292
Mycn                  0.009986          0.055351
E2f4                  0.011874          0.333948
Zbtb1                  0.000000          0.040590
Ybx1                  0.027709          0.127306

      degree_centrality_in  degree_centrality_out  betweenness_centrality \
Stat3          0.000000          0.151292              0
Mycn          0.000000          0.055351              0
E2f4          0.003690          0.330258            3158
Zbtb1          0.000000          0.040590              0
Ybx1          0.016605          0.110701            1051

      closeness_centrality  eigenvector_centrality  page_rank \

```

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Stat3	0.000012	0.487978	0.001633	
Mycn	0.000010	0.245650	0.001633	
E2f4	0.000010	1.000000	0.001724	
Zbtb1	0.000004	0.113727	0.001633	
Ybx1	0.000004	0.385376	0.002133	
 assortative_coefficient average_path_length community_random_walk \				
Stat3	-0.161693	2.621324	1	
Mycn	-0.161693	2.621324	1	
E2f4	-0.161693	2.621324	1	
Zbtb1	-0.161693	2.621324	18	
Ybx1	-0.161693	2.621324	2	
 module connectivity participation role cluster				
Stat3	0	3.573858	Connector Hub	Ery_0
Mycn	2	1.767680	Peripheral	Ery_0
E2f4	0	8.575037	Connector Hub	Ery_0
Zbtb1	2	1.317952	Peripheral	Ery_0
Ybx1	3	5.306800	Connector Hub	Ery_0

## 6.4. Save

Save processed GRN. We use this file in the next notebook; “in silico perturbation with GRNs”.

```
[31]: # Save Links object.
links.to_hdf5(file_path="links.celloracle.links")
```

```
[6]: # You can load files with the following command.
links = co.load_hdf5(file_path="links.celloracle.links")
```

## 7. Network analysis; Network score for each gene

The Links class has many functions to visualize network score. See the documentation for the details of the functions.

### 7.1. Network score in each cluster

We have calculated several network scores using different centrality metrics. We can use the centrality score to identify key regulatory genes because centrality is one of the important indicators of network structure (<https://en.wikipedia.org/wiki/Centrality>).

Let’s visualize genes with high network centrality.

```
[83]: # Check cluster name
links.cluster
```

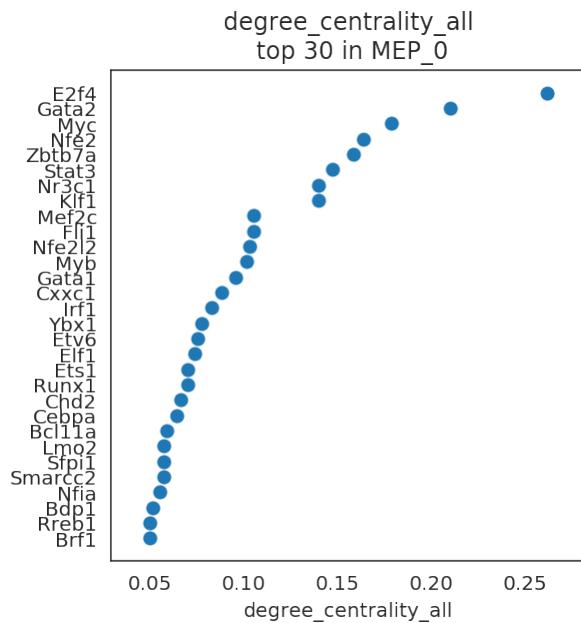
```
[83]: ['Ery_0',
'Ery_1',
'Ery_2',
'Ery_3',
'Ery_4',
'Ery_5',
```

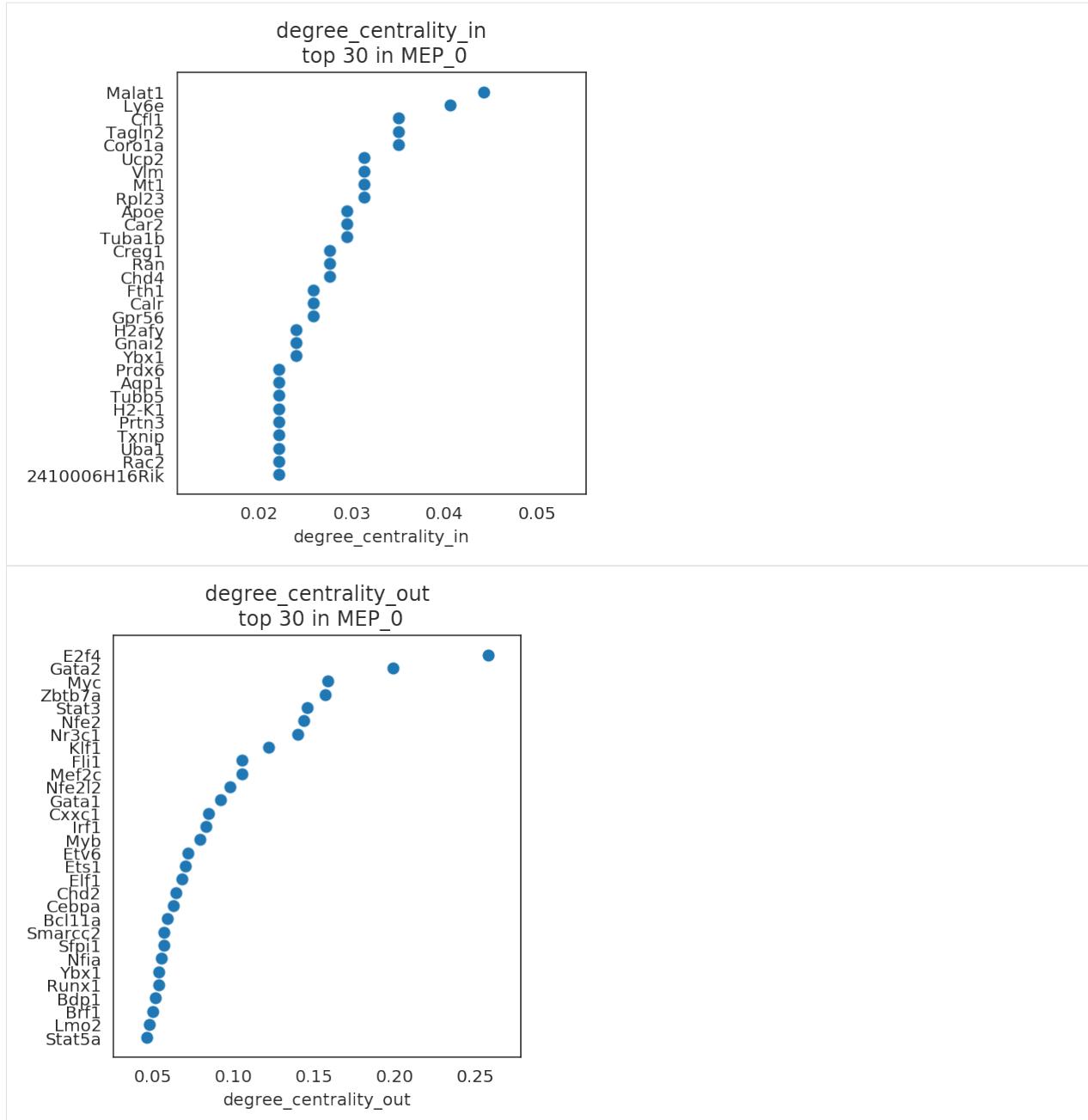
(continues on next page)

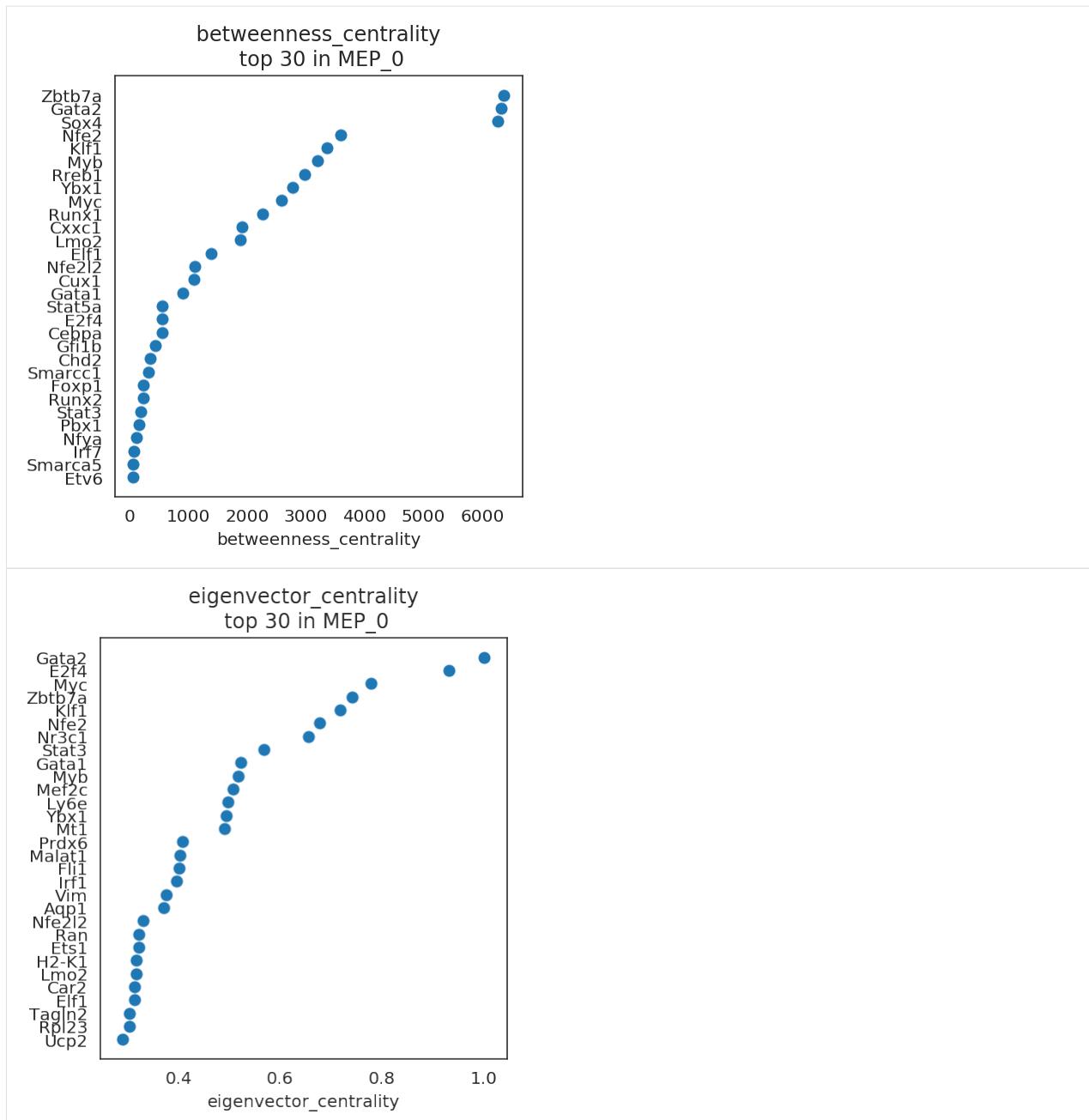
(continued from previous page)

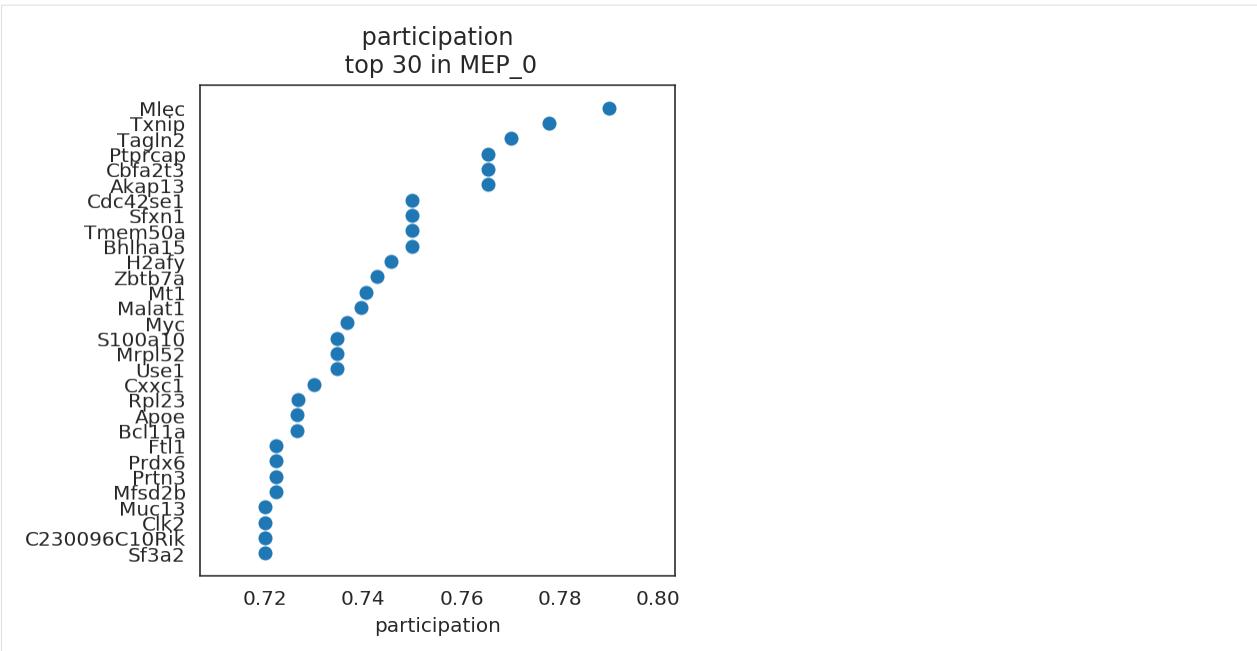
```
'Ery_6',
'Ery_7',
'Ery_8',
'Ery_9',
'GMP_0',
'GMP_1',
'GMP1_0',
'Gran_0',
'Gran_1',
'Gran_2',
'MEP_0',
'Mk_0',
'Mo_0',
'Mo_1']
```

[53]: # Visualize top n-th genes that have high scores.  
links.plot\_scores\_as\_rank(cluster="MEP\_0", n\_gene=30, save=f"{save\_folder}/ranked\_
→score")





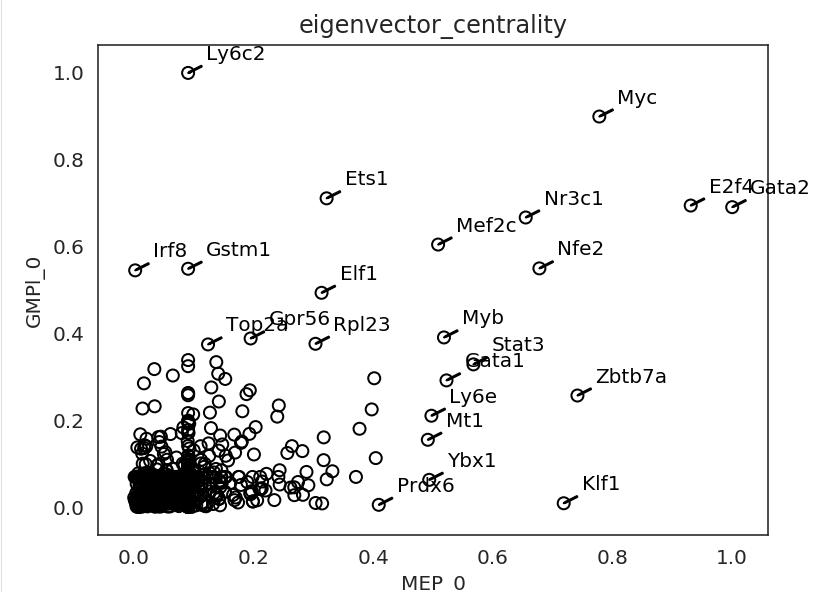




## 7.2. Network score comparison between two clusters

By comparing network scores between two clusters, we can analyze differences in GRN structure.

```
[54]: plt.ticklabel_format(style='sci',axis='y',scilimits=(0,0))
links.plot_score_comparison_2D(value="eigenvector_centrality",
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f'{save_folder}/score_comparison')
```

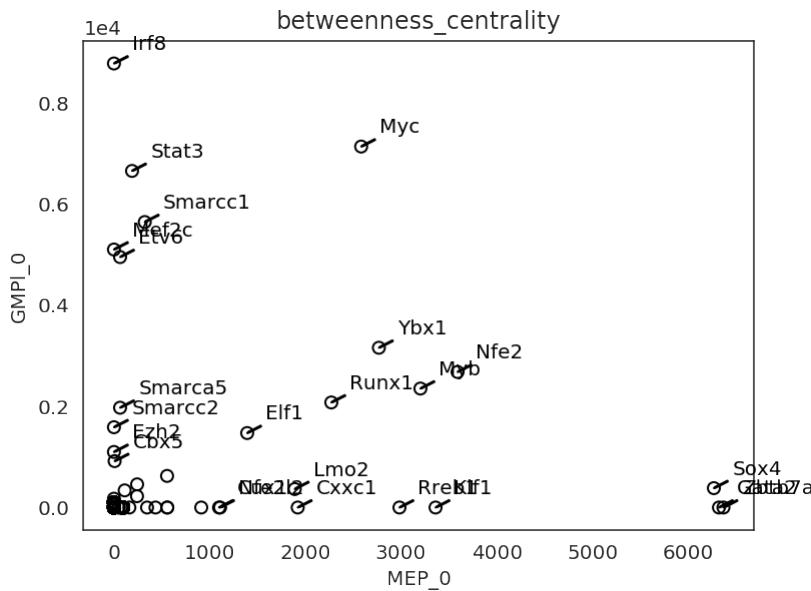


```
[55]: plt.ticklabel_format(style='sci',axis='y',scilimits=(0,0))
```

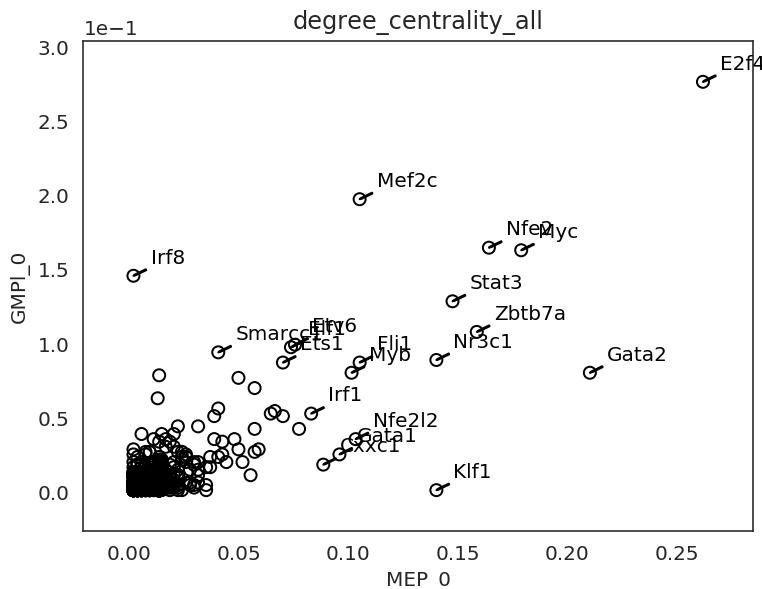
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```
links.plot_score_comparison_2D(value="betweenness_centrality",
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f"{save_folder}/score_comparison")
```



```
[56]: plt.ticklabel_format(style='sci', axis='y', scilimits=(0,0))
links.plot_score_comparison_2D(value="degree_centrality_all",
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f"{save_folder}/score_comparison")
```

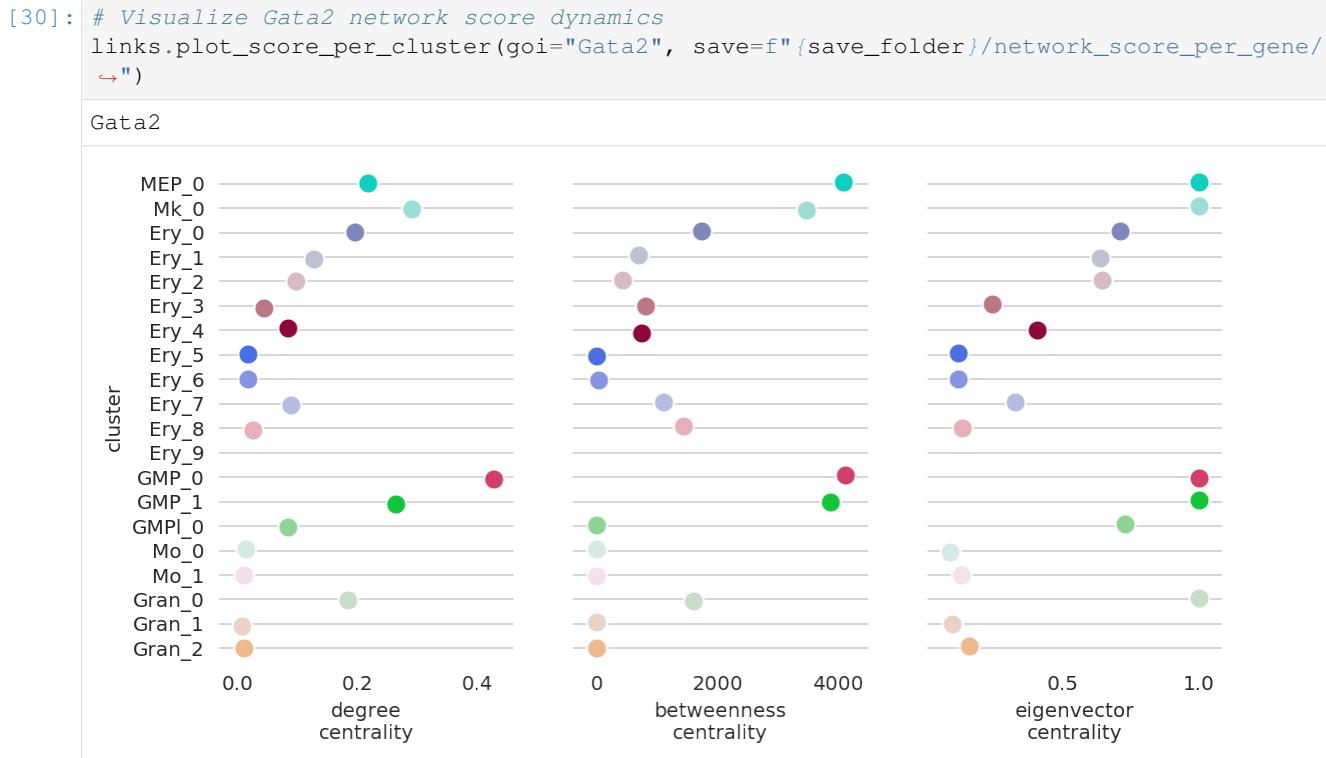


### 7.3. Network score dynamics

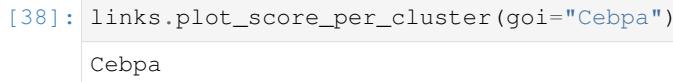
In the following session, we focus on how a gene's network score changes during the differentiation.

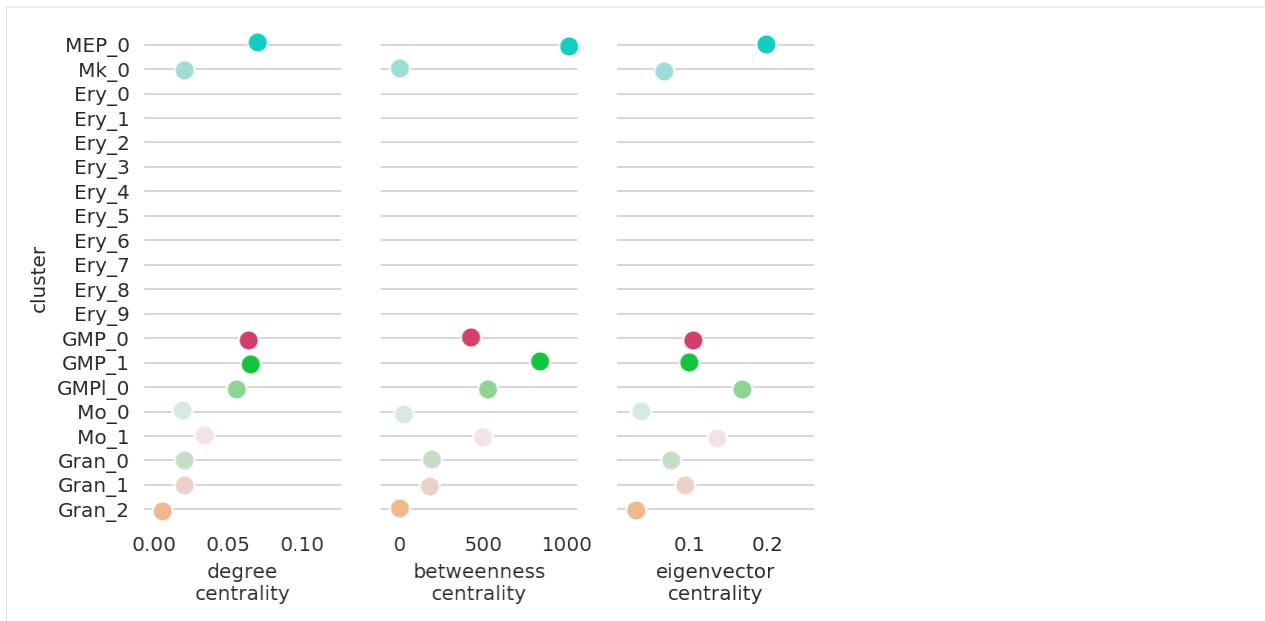
Using Gata2, we will demonstrate how you can visualize networks scores for a single gene.

Gata2 is known to play an essential role in the early MEP and GMP populations. .



If a gene have no connections in a cluster, it is impossible to calculate network degree scores. Thus the scores will not be shown. For example, Cebpa have no connection in the erythroids clusters, and there is no degree scores for Cebpa in these clusters as follows.





You can check filtered network edge as follows.

```
[39]: cluster_name = "Ery_0"
filtered_links_df = links.filtered_links[cluster_name]
filtered_links_df.head()
```

	source	target	coef_mean	coef_abs	p	-logp
68775	Stat3	Top2a	-0.107635	0.107635	1.976987e-14	13.703996
51655	Mycn	Prdx6	-0.096651	0.096651	8.076169e-11	10.092795
41345	Mycn	Mt1	-0.093897	0.093897	8.228218e-15	14.084694
5136	Ybx1	Anp32b	0.089403	0.089403	4.498303e-14	13.346951
41326	E2f4	Mt1	0.089261	0.089261	7.447929e-10	9.127964

You can confirm that there is no Cebpa connection in Ery\_0 cluster.

```
[41]: filtered_links_df[filtered_links_df.source == "Cebpa"]
```

	source	target	coef_mean	coef_abs	p	-logp
	Empty DataFrame					
	Columns:	source, target, coef_mean, coef_abs, p, -logp				
	Index:	[]				

## 7.4. Gene cartography analysis

Gene cartography is a method for gene network analysis. The method classifies gene into several groups using the network module structure and connections. It provides us an insight about the role and regulatory mechanism for each gene. For more information on gene cartography, please refer to the following paper (<https://www.nature.com/articles/nature03288>).

The gene cartography will be calculated for the GRN in each cluster. Thus we can know how the gene cartography change by comparing the the score between clusters.

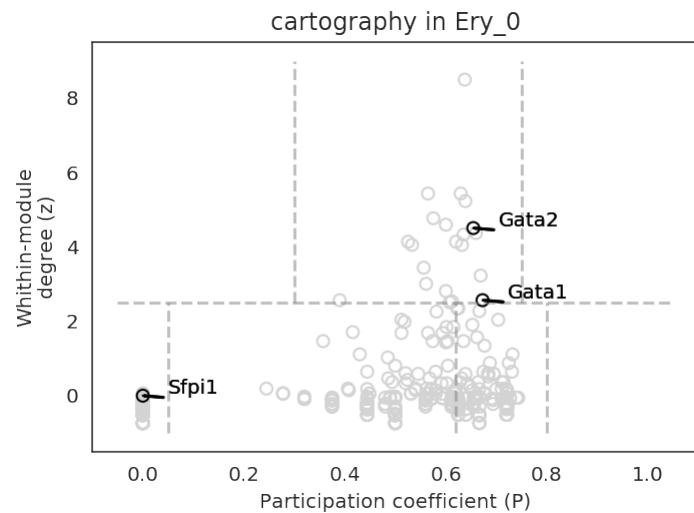
```
[58]: # Plot cartography as a scatter plot
links.plot_cartography_scatter_per_cluster(scatter=True,
                                            kde=False,
                                            gois=["Gata1", "Gata2", "Sfpil1"],
```

(continues on next page)

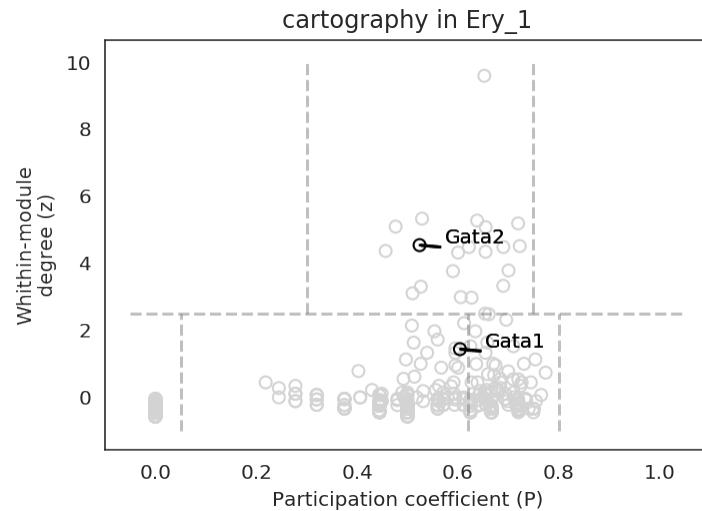
(continued from previous page)

```
auto_gene_annot=False,
args_dot={"n_levels": 105},
args_line={"c":"gray"}, save=f" {save_
→folder}/cartography")
```

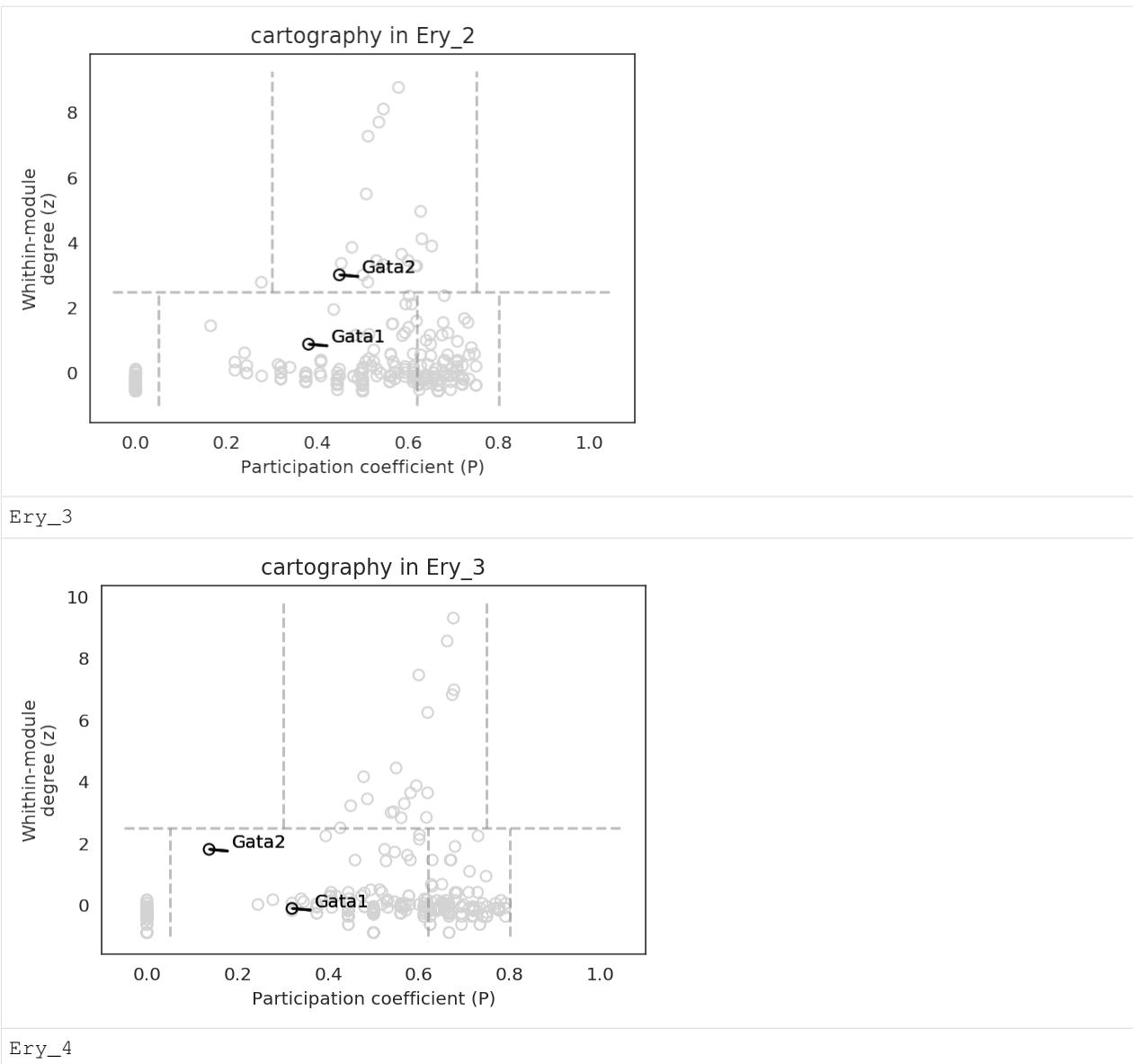
Ery\_0

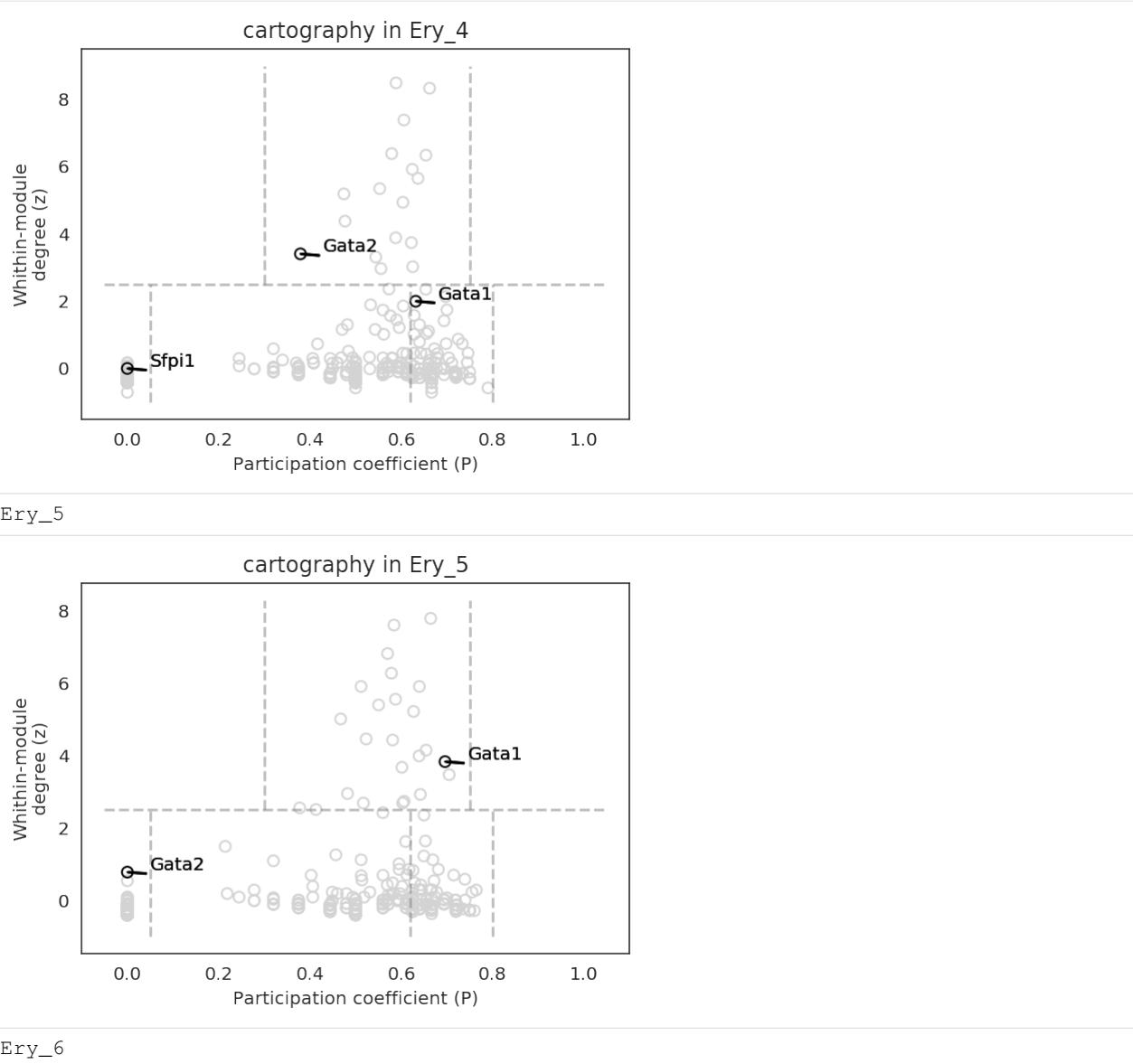


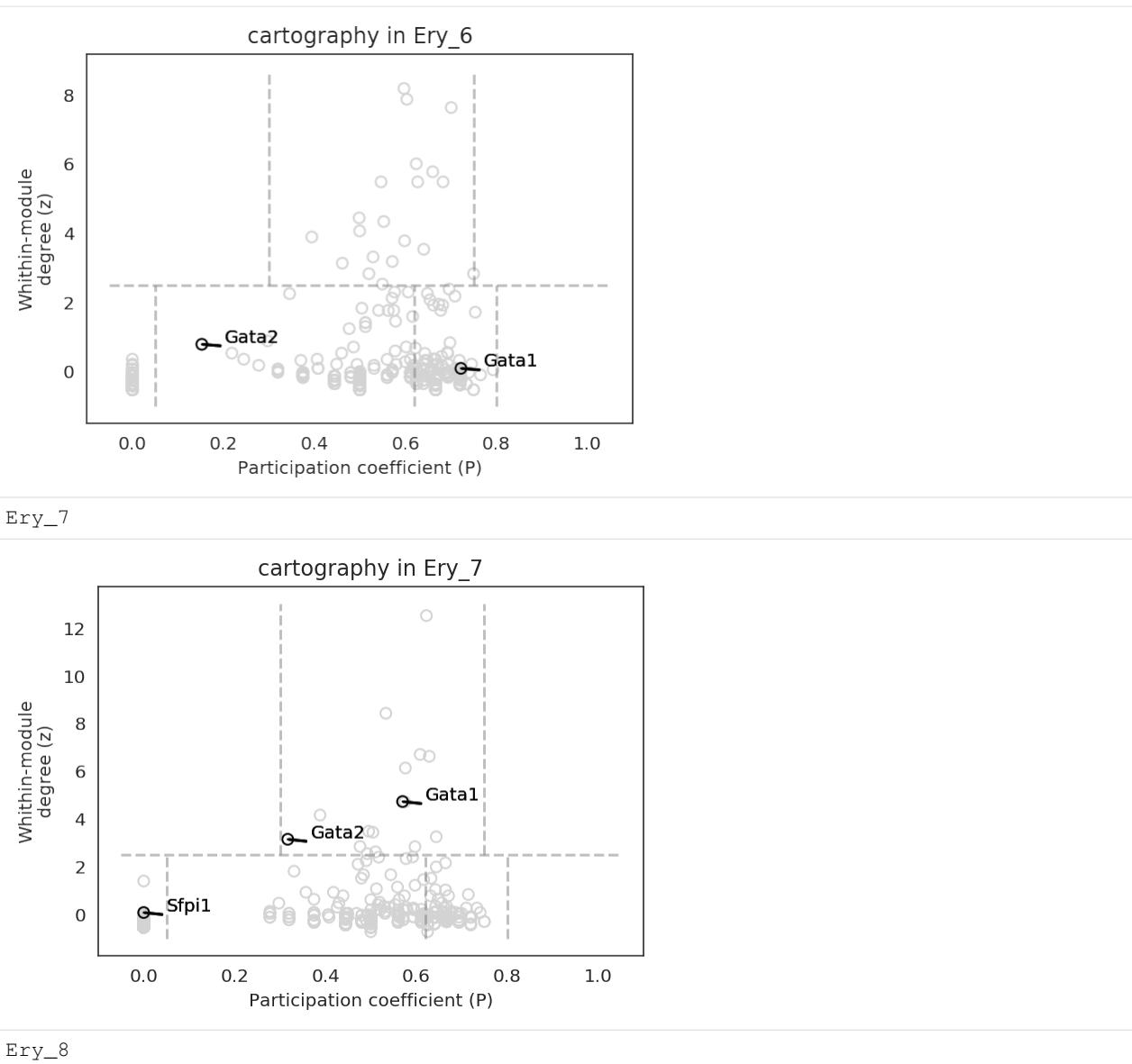
Ery\_1

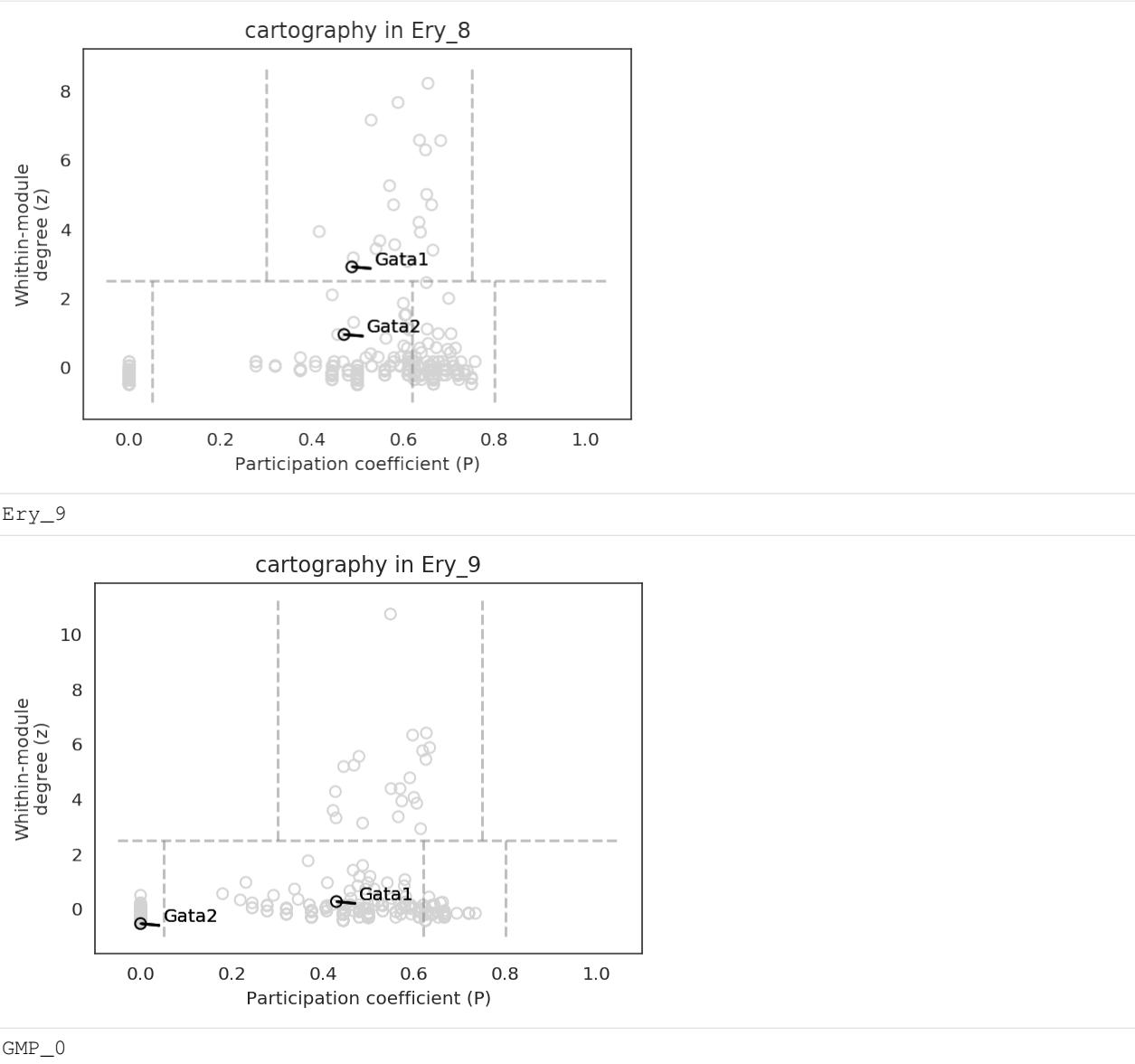


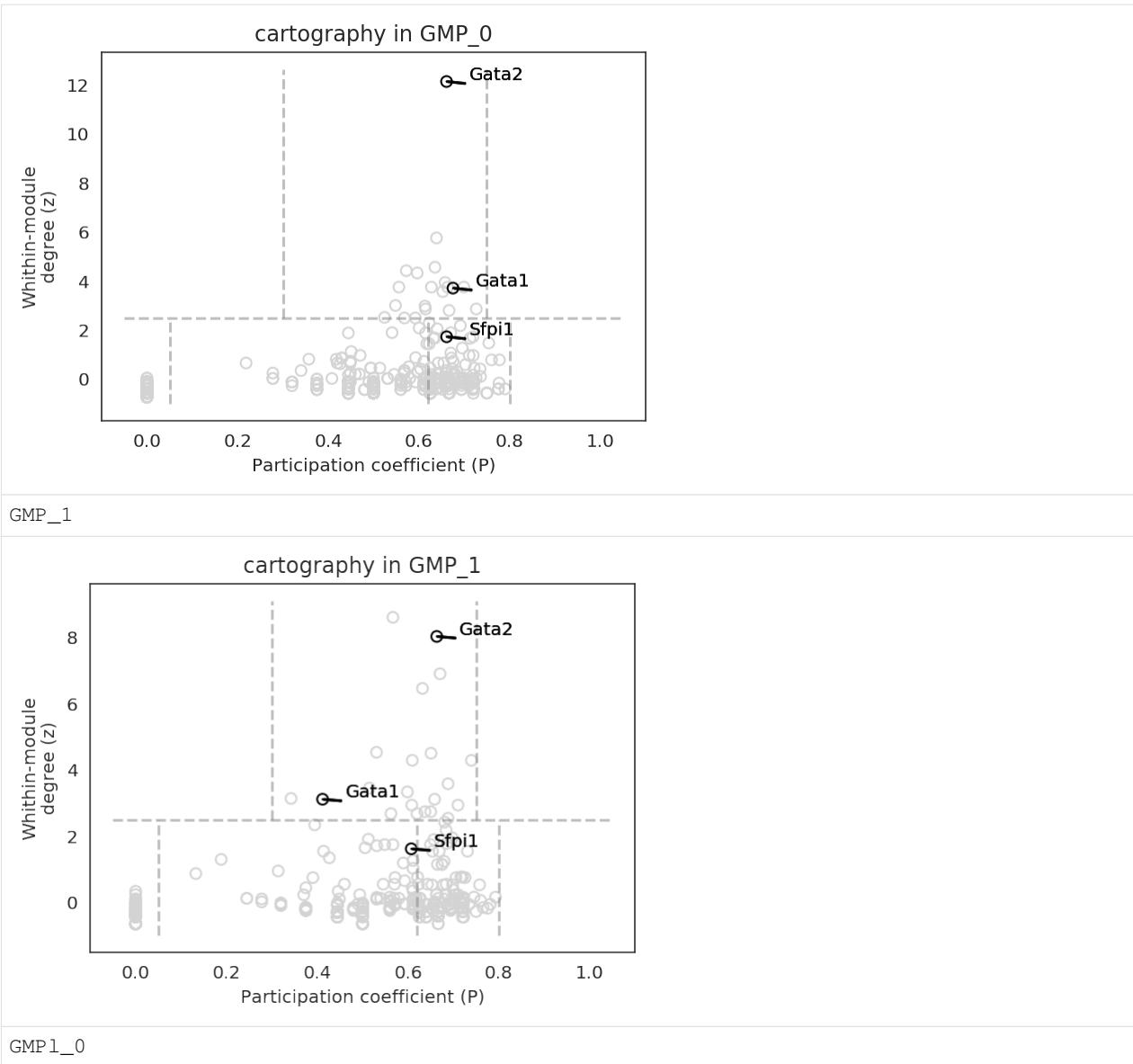
Ery\_2

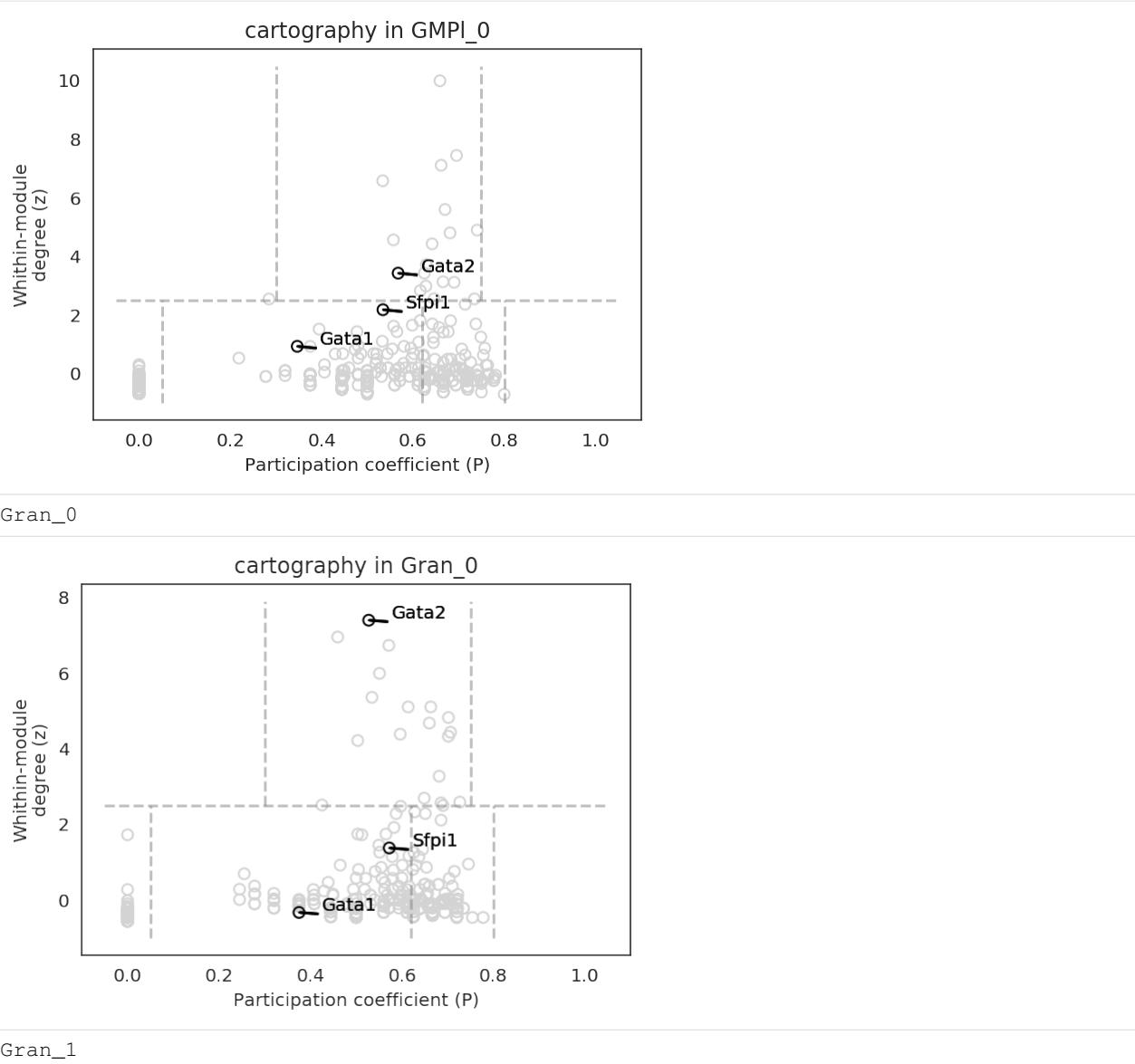


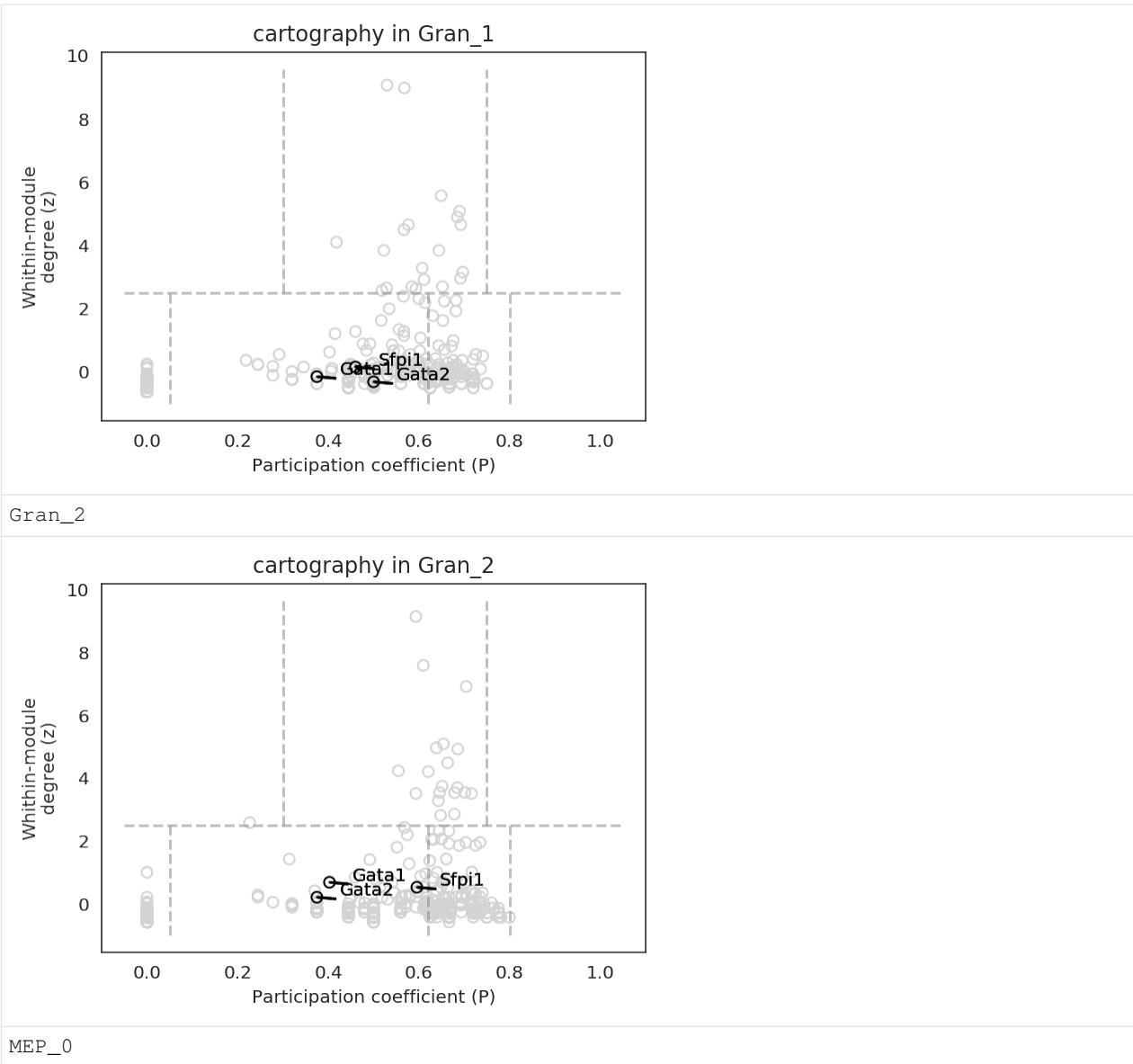


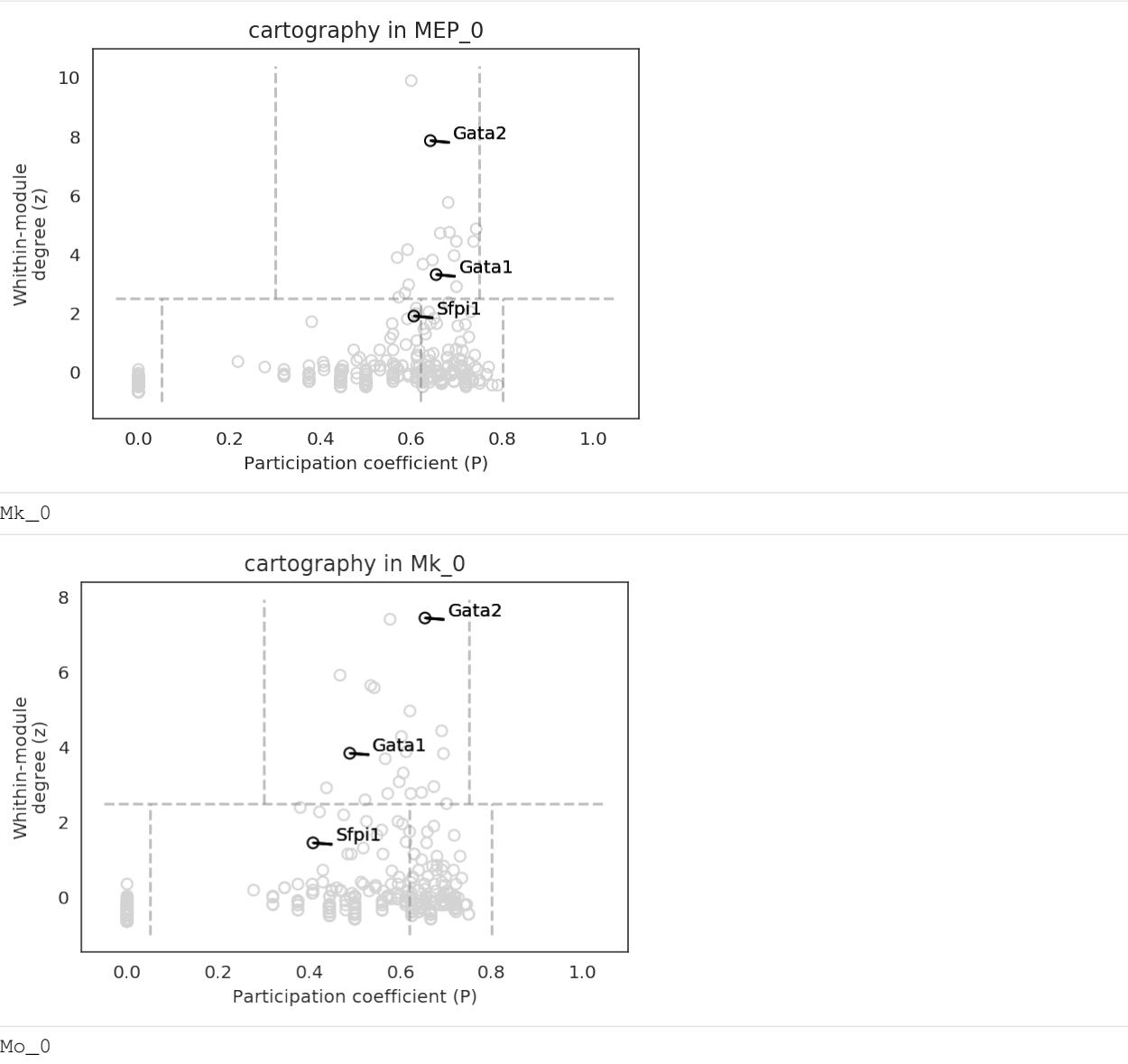


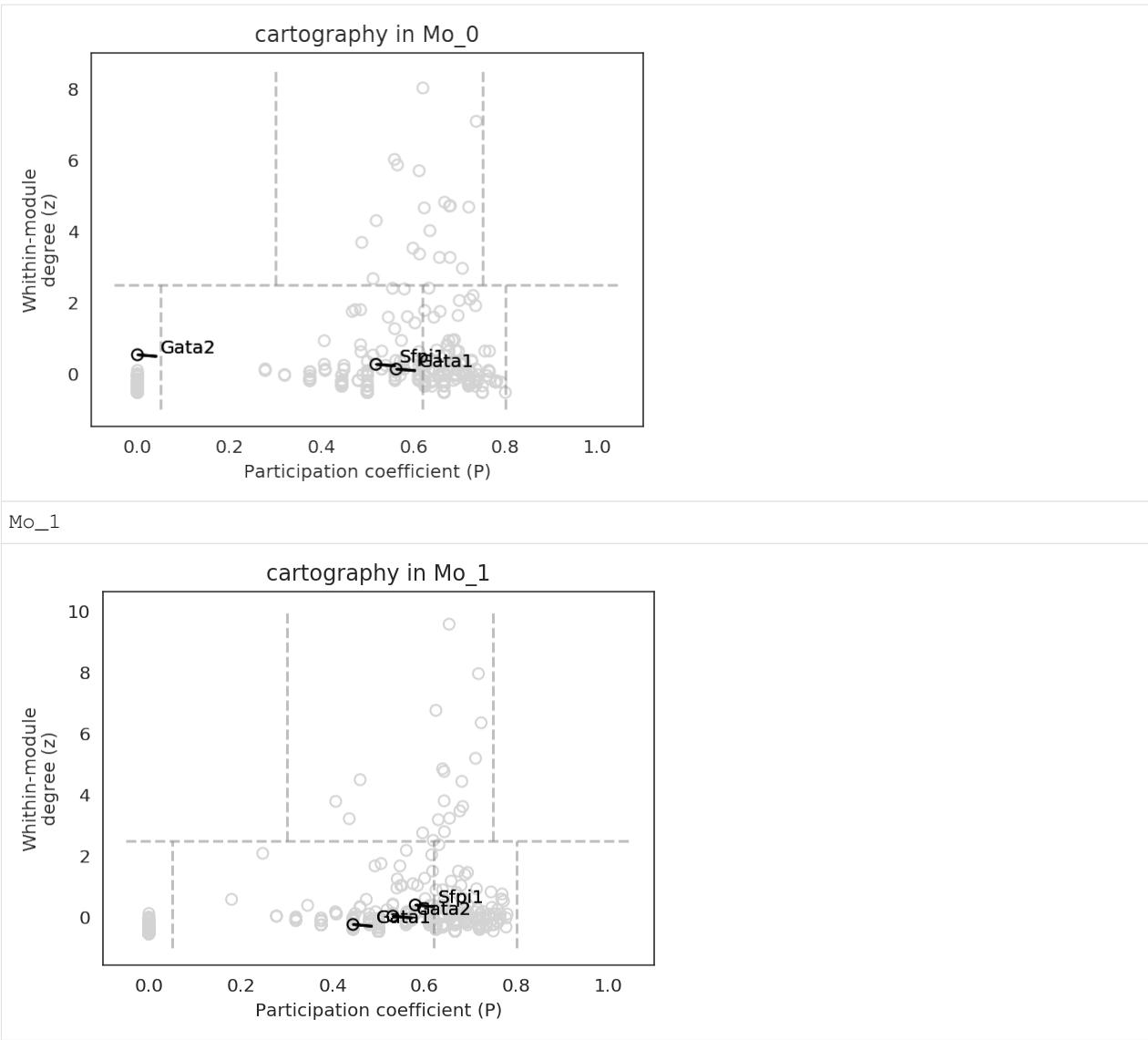








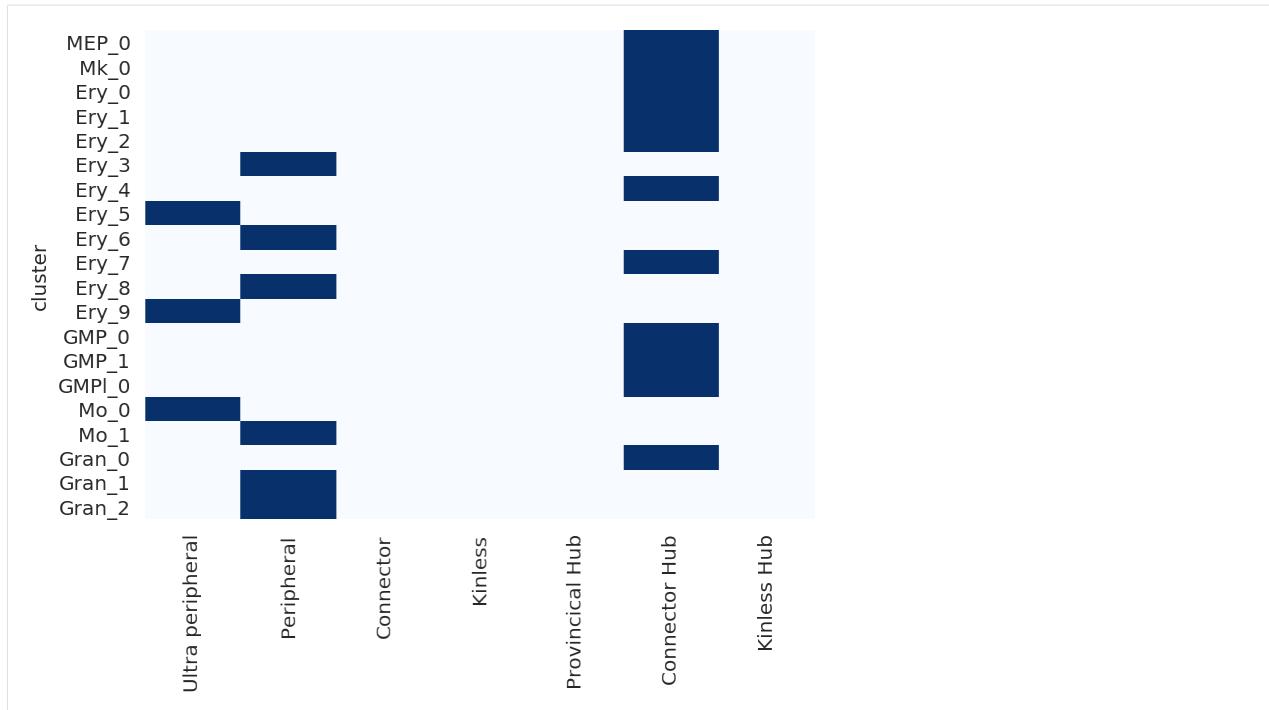




```
[66]: # Plot the summary of cartography analysis
```

```
links.plot_cartography_term(goi="Gata2", save=f"{save_folder}/cartography")
```

```
Gata2
```



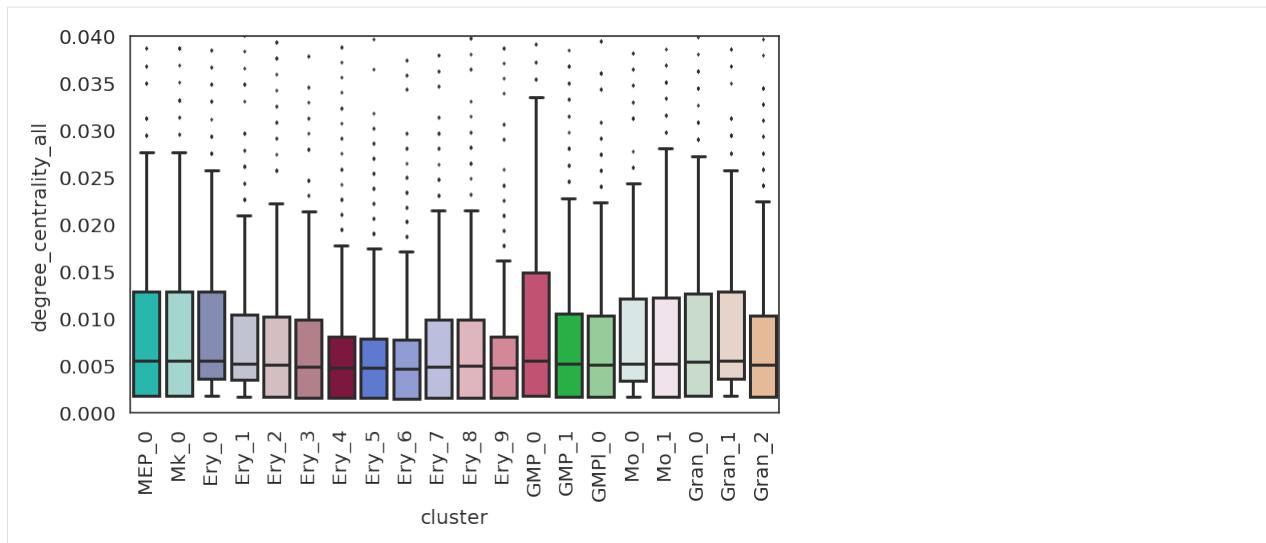
## 8. Network analysis; network score distribution

Next, we visualize the distribution of network score to get insight into the global trend of the GRNs.

### 8.1. Distribution of network degree

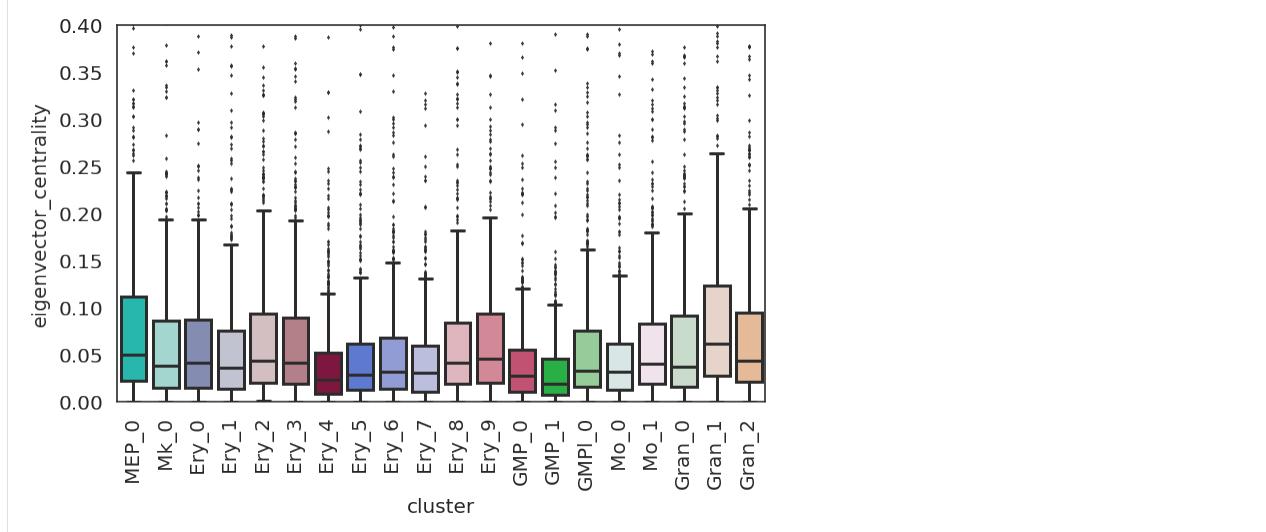
```
[60]: plt.subplots_adjust(left=0.15, bottom=0.3)
plt.ylim([0,0.040])
links.plot_score_distributions(values=["degree_centrality_all"], method="boxplot",
                                save=f"{save_folder}")
```

degree\_centrality\_all



```
[61]: plt.subplots_adjust(left=0.15, bottom=0.3)
plt.ylim([0, 0.40])
links.plot_score_distributions(values=["eigenvector_centrality"], method="boxplot",
                               save=f'{save_folder}'")
```

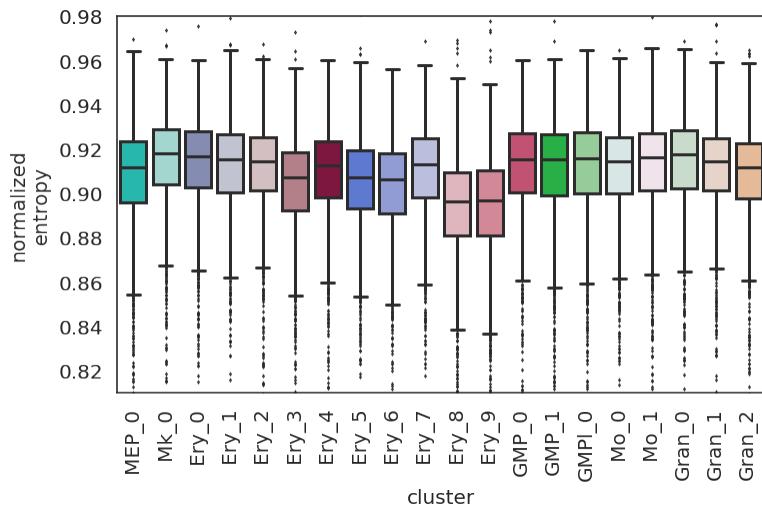
eigenvector\_centrality



## 8.2. Distribution of network entropy

```
[62]: plt.subplots_adjust(left=0.15, bottom=0.3)
links.plot_network_entropy_distributions(save=f"/{save_folder}/")
```

```
/home/k/anaconda3/envs/test/lib/python3.6/site-packages/scipy/stats/_distn_infrastructure.py:2614: RuntimeWarning: invalid value encountered in true_divide
  pk = 1.0*pk / np.sum(pk, axis=0)
/home/k/anaconda3/envs/test/lib/python3.6/site-packages/celloracle/network_analysis/links_object.py:345: RuntimeWarning: divide by zero encountered in log
    ent_norm.append(en/np.log(k[i]))
/home/k/anaconda3/envs/test/lib/python3.6/site-packages/celloracle/network_analysis/links_object.py:345: RuntimeWarning: invalid value encountered in double_scalars
    ent_norm.append(en/np.log(k[i]))
```



Using the network scores, we could pick up cluster-specific key TFs. Gata2, Gata1, Klf1, E2f1, for example, are known to play an essential role in MEP, and these TFs showed high network score in our GRN.

However, it is important to note that network analysis alone cannot shed light on the specific functions or roles these TFs play in cell fate determination.

In the next section, we will begin to investigate each TF's contribution to cell fate by running GRN simulations

```
[ ]:
```

### 1.2.5 Simulation with GRNs

celloracle leverage GRNs to simulate signal propagation inside a cell. We can estimate the effect of gene perturbation by the simulation with GRNs.

Additionally, we will combine the signal propagation simulation with a cell state transition simulation. The latter simulation is performed by a python library for RNA-velocity analysis, called `velocyto`. This analysis may provide an insight into a complex system how TF controls enormous target genes to determine cell fate.

The jupyter notebook files and data used in this tutorial are available [here](#).

Python notebook

## 0. Import libraries

### 0.1. Import public libraries

```
[1]: import os
import sys

import matplotlib.colors as colors
import matplotlib.pyplot as plt
import numpy as np
import pandas as pd
import scanpy as sc
import seaborn as sns
```

```
[2]: import celloracle as co
```

```
[3]: plt.rcParams["font.family"] = "arial"
plt.rcParams["figure.figsize"] = [9, 6]
%config InlineBackend.figure_format = 'retina'
plt.rcParams["savefig.dpi"] = 600

%matplotlib inline
```

### 0.1. Make a folder to save graph

```
[5]: # Make folder to save plots
save_folder = "figures"
os.makedirs(save_folder, exist_ok=True)
```

## 1. Load data

### 1.1. Load processed oracle object

Load the oracle object. See the previous notebook for the notes on how to prepare the oracle object.

```
[7]: oracle = co.load_hdf5("../04_Network_analysis/Paul_15_data.celloracle.oracle")
```

### 1.2. Load inferred GRNs

In the previous notebook, we calculated GRNs. Now, we will use these GRNs for simulation. We import GRNs which were saved in the Links object.

```
[8]: links = co.load_hdf5("../04_Network_analysis/links.celloracle.links")
```

## 2. Make predictive models for simulation

We will fit ridge regression models again. This process takes less time than the GRN inference in the previous notebook because we only use significant TFs to predict target gene instead of all regulatory candidate TFs.

```
[12]: links.filter_links()
oracle.get_cluster_specific_TFdict_from_Links(links_object=links)
oracle.fit_GRN_for_simulation(alpha=10, use_cluster_specific_TFdict=True)

calculating GRN using cluster specific TF dict...
calculating GRN in Ery_0

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1074 genes
calculating GRN in Ery_1

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1092 genes
calculating GRN in Ery_2

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1064 genes
calculating GRN in Ery_3

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1105 genes
calculating GRN in Ery_4

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1102 genes
calculating GRN in Ery_5

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1116 genes
calculating GRN in Ery_6

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1097 genes
calculating GRN in Ery_7

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
```

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```
models made for 1062 genes
calculating GRN in Ery_8
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1117 genes
calculating GRN in Ery_9
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1121 genes
calculating GRN in GMP_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1107 genes
calculating GRN in GMP_1
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1104 genes
calculating GRN in GMPl_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1089 genes
calculating GRN in Gran_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1067 genes
calculating GRN in Gran_1
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1076 genes
calculating GRN in Gran_2
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1105 genes
calculating GRN in MEP_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1152 genes
calculating GRN in Mk_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1114 genes
calculating GRN in Mo_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1085 genes
calculating GRN in Mo_1
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1074 genes
```

### 3. in silico Perturbation-simulation

Next, we will simulate the effects of perturbing a single TF to investigate its function and regulatory mechanism. See the celloracle paper for the details and scientific premise on the algorithm.

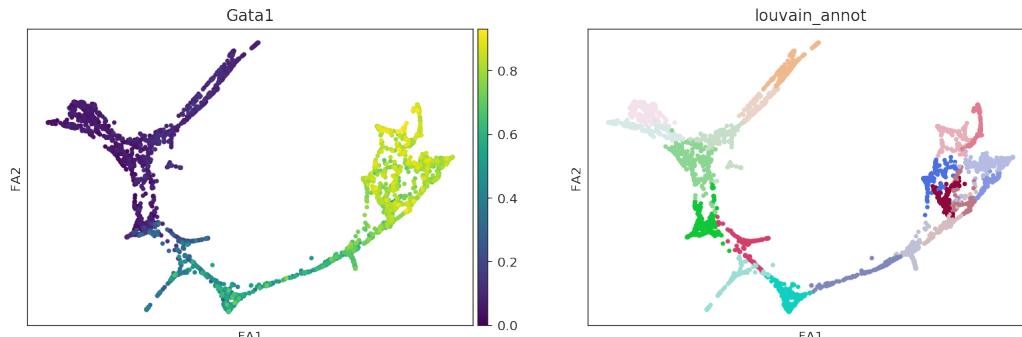
In this notebook, we'll show an example of the simulation; we'll simulate knock-out of Gata1 gene in the hematopoiesis.

Previous studies have shown that Gata1 is one of the TFs that regulates cell fate decisions in myeloid progenitors. Additionally, Gata1 has been shown to affect erythroid cell differentiation.

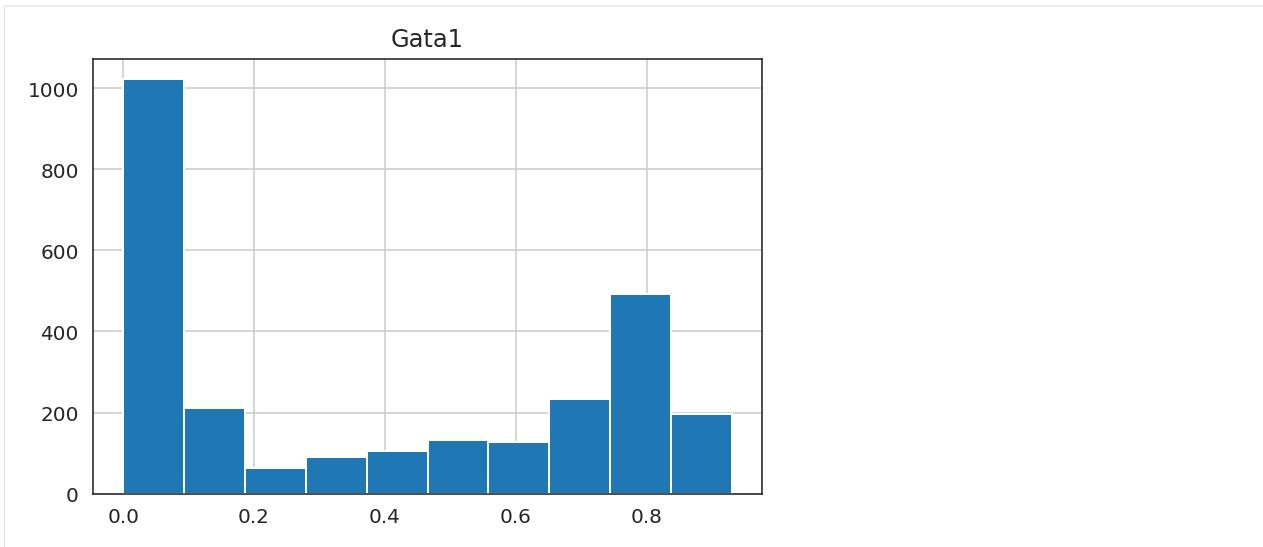
Here, we will analyze Gata1 for the demonstration of celloracle; Celloracle try to recapitulate the previous findings of Gata1 gene above.

#### 3.1. Check gene expression pattern.

```
[26]: # Check gene expression
goi = "Gata1"
sc.pl.draw_graph(oracle.adata, color=[goi, oracle.cluster_column_name],
                 layer="imputed_count", use_raw=False, cmap="viridis")
```



```
[33]: # Plot gene expression in histogram
sc.get.obs_df(oracle.adata, keys=[goi], layer="imputed_count").hist()
plt.show()
```



### 3.2. calculate future gene expression after perturbation.

Although you can use any gene expression value for the input of in silico perturbation, we recommend avoiding extreme values which are far from natural gene expression ranges. If you set Gata1 gene expression to 100, for example, it may lead to biologically infeasible results.

Here we simulate Gata1 KO; we predict what happens to the cells if Gata1 gene expression changed into 0.

```
[34]: # Enter perturbation conditions to simulate signal propagation after the perturbation.
oracle.simulate_shift(perturb_condition={goi: 0.0},
                      n_propagation=3)
```

### 3.3. calculate transition probability between cells

In the step above, we simulated simulated future gene expression values after perturbation. This prediction is based on iterative calculations of signal propagations within the GRN.

Next step, we will calculate the probability of a cell state transition based on the simulated data. Using the transition probability between cells, we can predict how a cell changes after perturbation.

This transition probability will be used in two ways.

- (1) Visualization of directed trajectory graph.
- (2) Markov simulation.

In Step 4.2 and 4.3, we use functions imported from the velocytoloom class in velocyto.py. Please see the documentation of VelocytoLoom for more information. [http://velocyto.org/velocyto.py/fullapi/api\\_analysis.html](http://velocyto.org/velocyto.py/fullapi/api_analysis.html)

```
[35]: # Get transition probability
oracle.estimate_transition_prob(n_neighbors=200, knn_random=True, sampled_fraction=0.
                                ↵5)

# Calculate embedding
oracle.calculate_embedding_shift(sigma_corr = 0.05)
```

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```
# Calculate global trend of cell transition
oracle.calculate_grid_arrows(smooth=0.8, steps=(40, 40), n_neighbors=300)

/home/k/anaconda3/envs/test/lib/python3.6/site-packages/IPython/core/interactiveshell.py:3326: FutureWarning: arrays to stack must be passed as a "sequence" type such as
list or tuple. Support for non-sequence iterables such as generators is deprecated
as of NumPy 1.16 and will raise an error in the future.
exec(code_obj, self.user_global_ns, self.user_ns)
WARNING:root:Nans encountered in corrcoef and corrected to 1s. If not identical cells
were present it is probably a small isolated cluster converging after imputation.
```

## 4. Visualization

### 4.1. Detailed directed trajectory graph

```
[36]: plt.figure(None, (6, 6))
quiver_scale = 40

ix_choice = np.random.choice(oracle.adata.shape[0], size=int(oracle.adata.shape[0]/1.
), replace=False)

embedding = oracle.adata.obsm[oracle.embedding_name]

plt.scatter(embedding[ix_choice, 0], embedding[ix_choice, 1],
            c="0.8", alpha=0.2, s=38, edgecolor=(0,0,0,1), lw=0.3, rasterized=True)

quiver_kw_args=dict(headaxislength=7, headlength=11, headwidth=8,
                     linewidths=0.35, width=0.0045, edgecolors="k",
                     color=oracle.colorandum[ix_choice], alpha=1)
plt.quiver(embedding[ix_choice, 0], embedding[ix_choice, 1],
            oracle.delta_embedding[ix_choice, 0], oracle.delta_embedding[ix_choice, 1],
            scale=quiver_scale, **quiver_kw_args)

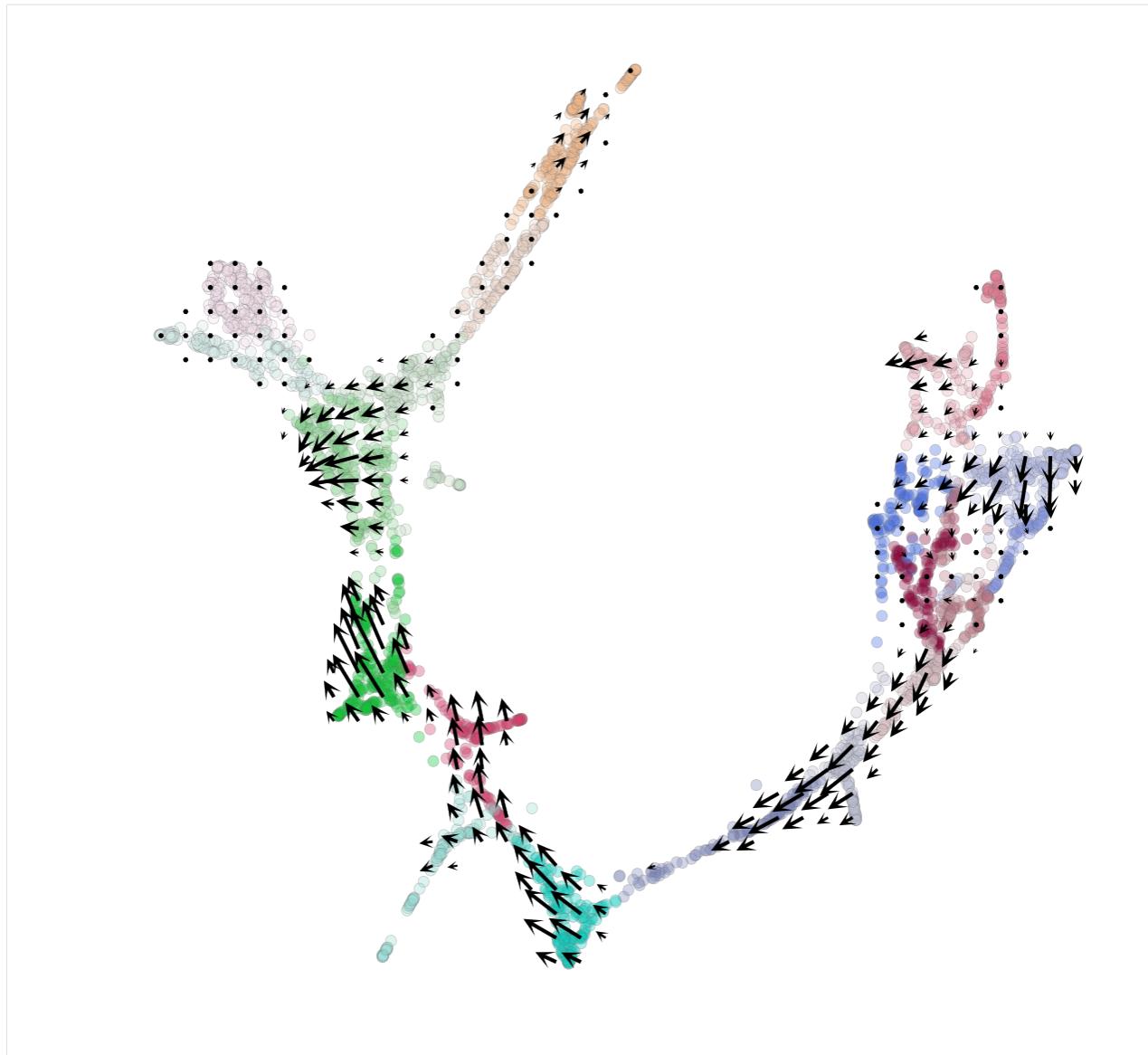
plt.axis("off")
# plt.savefig(f"{save_folder}/full_arrows(goi).png", transparent=True)

[36]: (-10815.27020913708, 10950.84121716522, -10711.36365432337, 10949.477199695968)
```



## 4.2. Grid graph

```
[37]: # Plot whole graph
plt.figure(None, (10,10))
oracle.plot_grid_arrows(quiver_scale=2.0,
                        scatter_kwarg_dict={"alpha":0.35, "lw":0.35,
                                            "edgecolor":"0.4", "s":38,
                                            "rasterized":True},
                        min_mass=0.015, angles='xy', scale_units='xy',
                        headaxislength=2.75,
                        headlength=5, headwidth=4.8, minlength=1.5,
                        plot_random=False, scale_type="relative")
# plt.savefig(f"{save_folder}/vectorfield_{goi}.png", transparent=True)
```



## 5. Markov simulation to analyze the effects of perturbation on cell fate transition

We can also simulate cell state transition using Markov simulation.

### 5.1. Do Markov simulation

We will simulate using the parameters, “n\_steps=200” and “n\_duplication=5” in the following example.

To elaborate, this means:

- (1) We will do 200 times of iterative simulations to predict how the cell changes over time
- (2) We will repeat 5 rounds of simulations

```
[83]: %time
# n_steps is the number of steps in markov simulation.
# n_duplication is the number of technical duplication for the simulation
oracle.run_markov_chain_simulation(n_steps=200, n_duplication=5)

CPU times: user 1.33 s, sys: 0 ns, total: 1.33 s
Wall time: 1.33 s
```

## 5.2. Check the results of the simulation for specific cells

Check the results of simulation. Pick up some cells and visualize their transition trajectory.

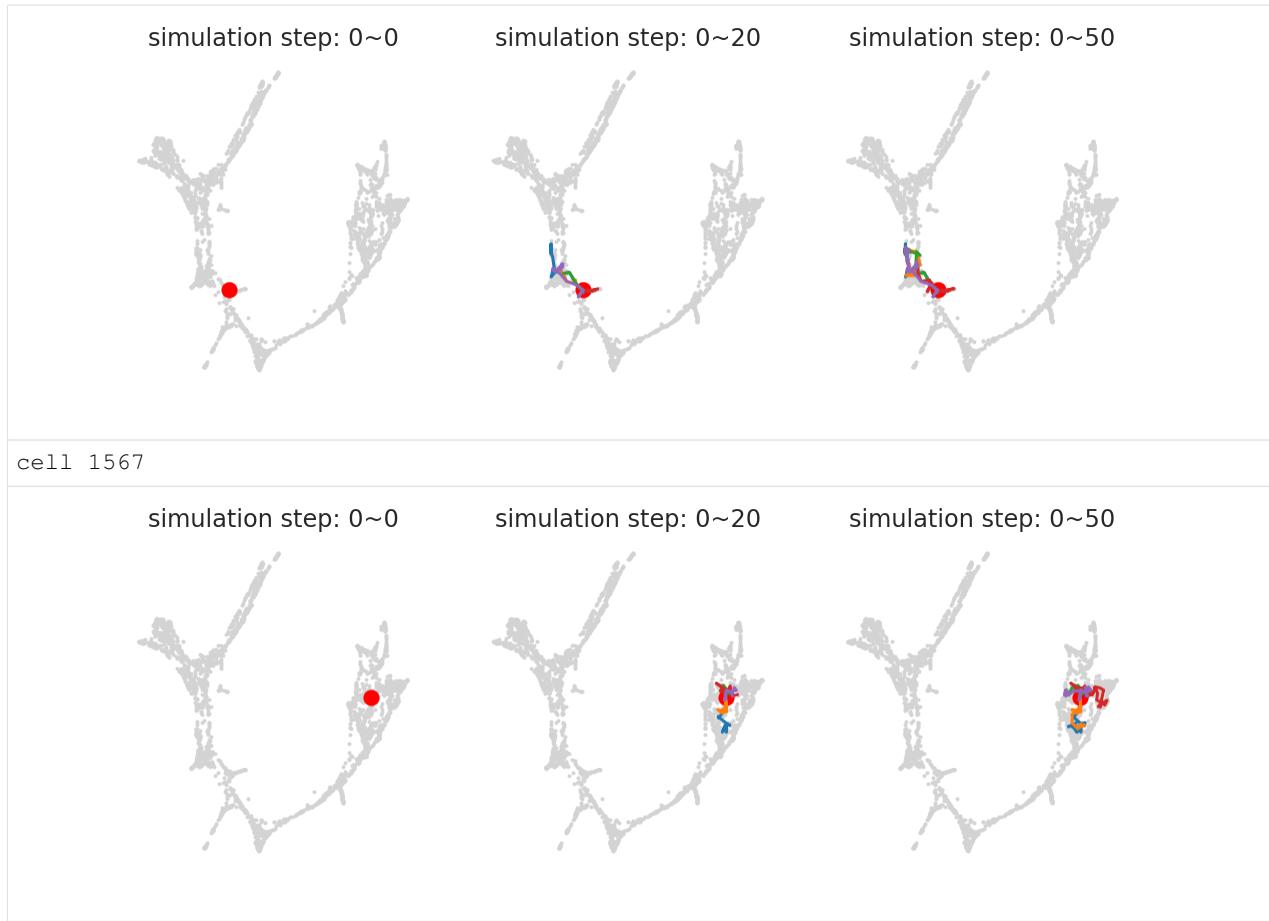
```
[88]: # Randomly pick up 3 cells
np.random.seed(12)
cells = oracle.adata.obs.index.values[np.random.choice(oracle.ixs_mcmc, 3)]

# Visualize the simulated results of cell transition after perturbation
for k in cells:
    print(f"cell {k}")
    plt.figure(figsize=[9, 3])
    for j, i in enumerate([0, 20, 50]): # time points
        plt.subplot(1, 3, (j+1))
        oracle.plot_mc_result_as_trajectory(k, range(0, i))
        plt.title(f"simulation step: 0~{i}")
        plt.axis("off")
    plt.show()
```

cell 1961



cell 43

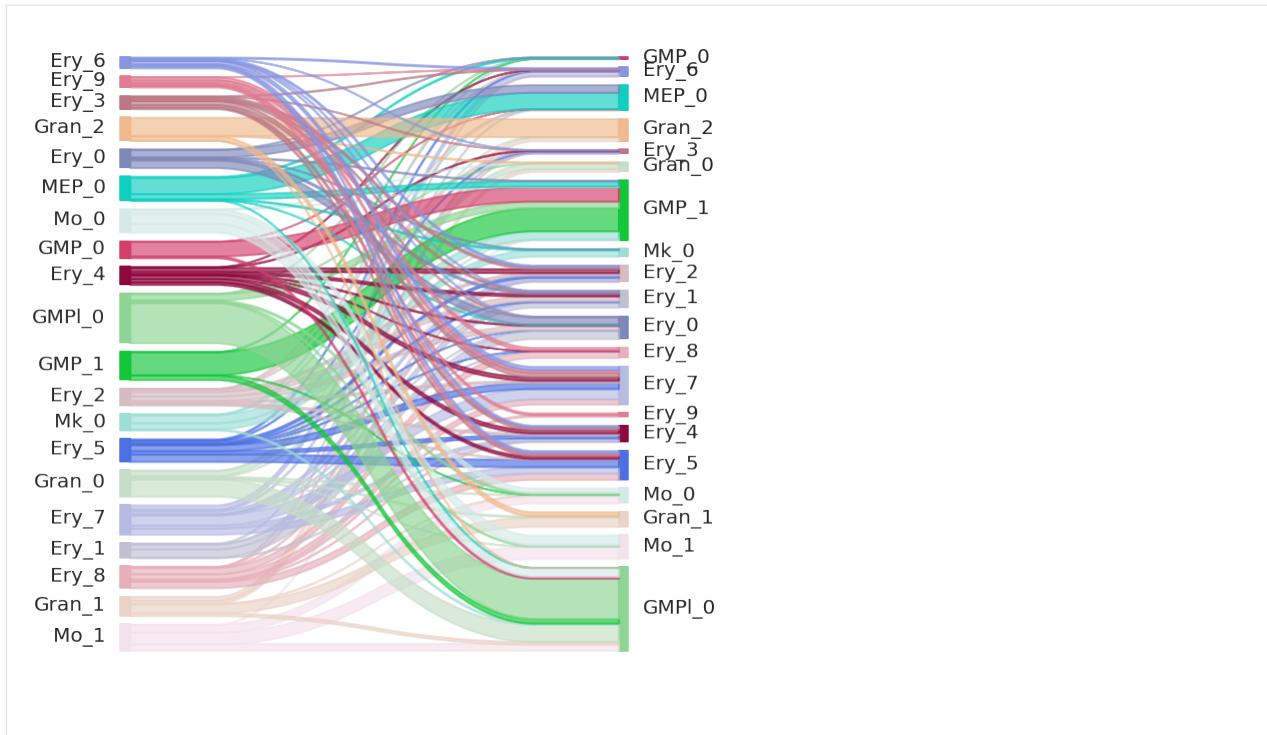


### 5.3. Summarize the results of simulation by plotting sankey diagram

Sankey diagrams are useful when you want to visualize proportional cell transitions between some groups.

For the grouping of cells, you can use arbitrary cluster unit.

```
[89]: # Plot sankey diagram
plt.figure(figsize=[5, 6])
cl = "louvain_annot"
oracle.plot_mc_results_as_sankey(cluster_use=cl, start=0, end=100)
```

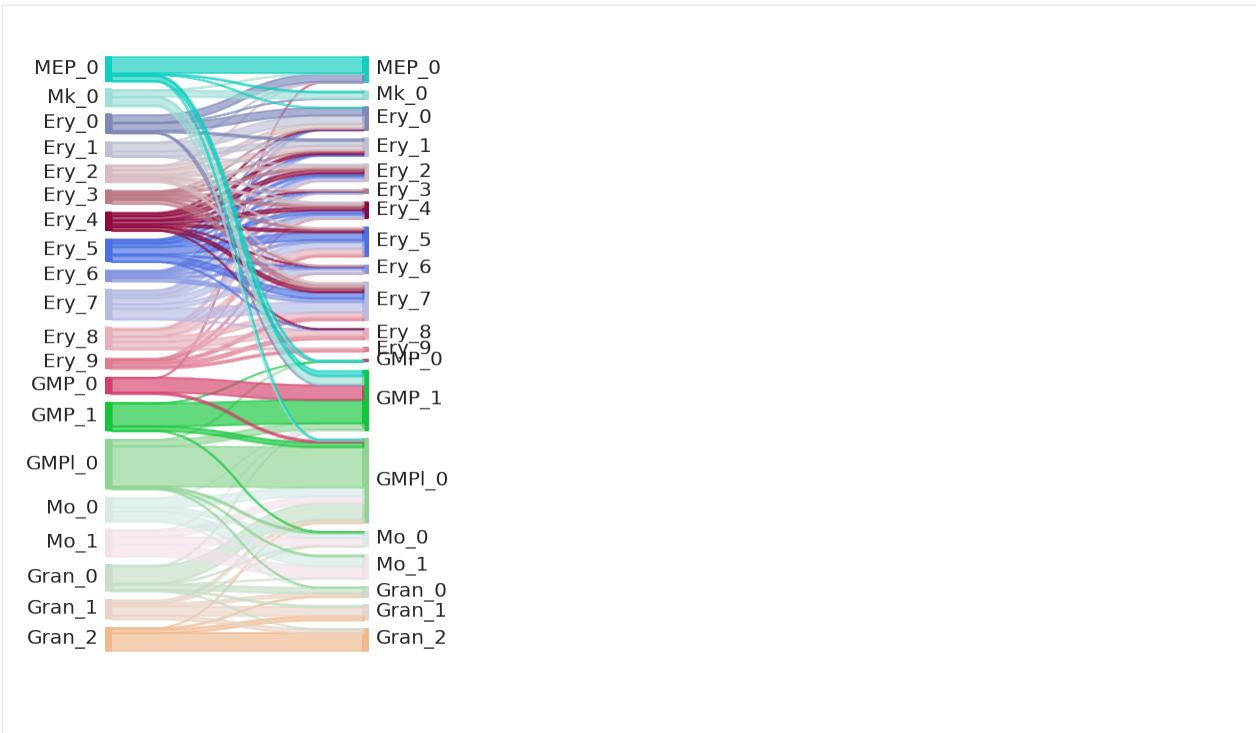


The Sankey diagram above looks messy because the cluster order is random.

Let's change the cluster order and make the plot again

```
[90]: cl = "louvain_annotation"
order = ['MEP_0', 'Mk_0', 'Ery_0', 'Ery_1', 'Ery_2', 'Ery_3', 'Ery_4',
         'Ery_5', 'Ery_6', 'Ery_7', 'Ery_8', 'Ery_9',
         'GMP_0', 'GMP_1', 'GMP_2', 'GMPI_0', 'GMPI_1',
         'Mo_0', 'Mo_1', 'Mo_2', 'Gran_0', 'Gran_1', 'Gran_2', 'Gran_3']

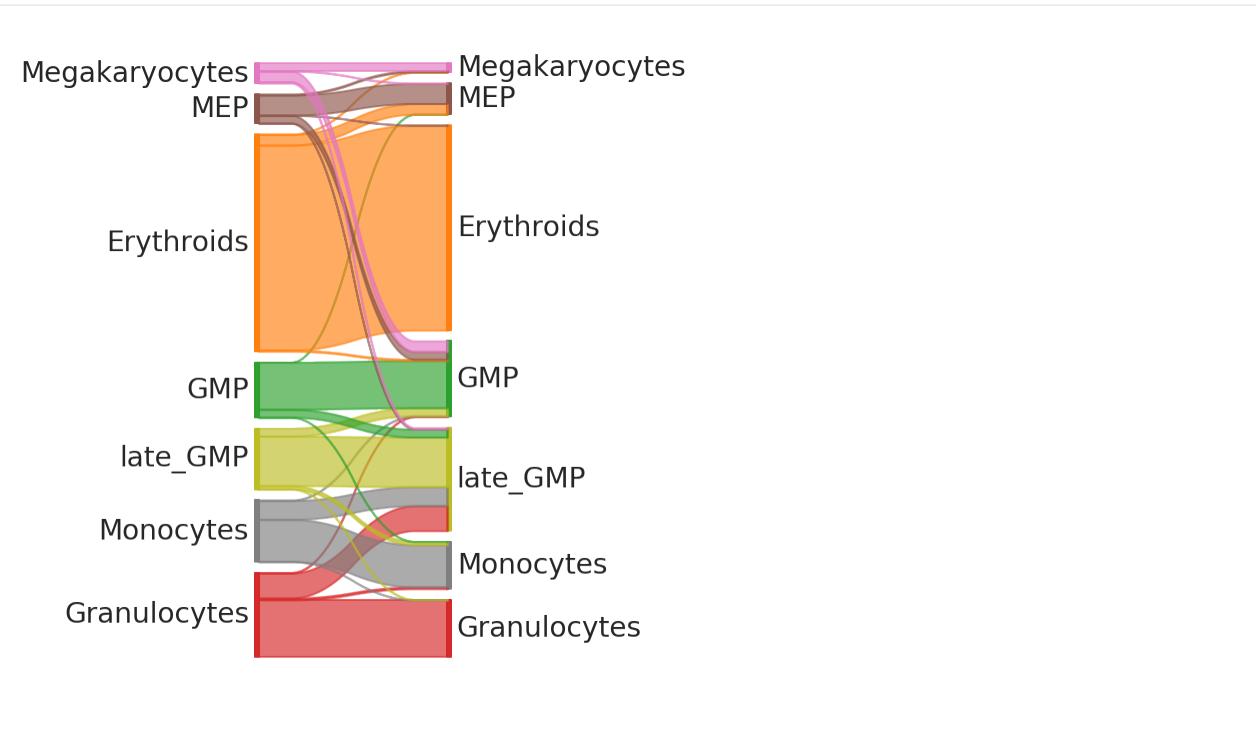
plt.figure(figsize=[5, 6])
plt.subplots_adjust(left=0.3, right=0.7)
oracle.plot_mc_results_as_sankey(cluster_use=cl, start=0, end=100, order=order)
# plt.savefig(f"{save_folder}/mcmc_{cl}.png")
```



Make another Saneky diagram with different cluster units.

```
[92]: order = ['Megakaryocytes', 'MEP', 'Erythroids', 'GMP', 'late_GMP', 'Monocytes',
   ↪'Granulocytes']
cl = "cell_type"

plt.figure(figsize=[5, 6])
plt.subplots_adjust(left=0.35, right=0.65)
oracle.plot_mc_results_as_sankey(cluster_use=cl, start=0, end=100, order=order, font_
   ↪size=14)
# plt.savefig(f"{save_folder}/mcmc_{cl}{goi}.png", transparent=True)
```



Based on the results, we may conclude several things as follows.

Gata1 KO induced both cell state transitions from Erythroids to MEP, and from MEP to GMP.

- (1) These results suggest that Gata1 may play a role in the progression of Erythroid differentiation and cell state determination between the MEP and GMP lineages.
- (2) Gata1 KO also induced cell state transitions from granulocytes to late GMP, suggesting Gata1's involvement in Granulocytes differentiation.

These results agree with previous reports about Gata1 and recapitulate Gata1's cell-type-specific function regarding the cell fate decisions in hematopoiesis.

## 1.3 API

### 1.3.1 Command Line API

CellOracle has a command line API. This command can be used to convert scRNA-seq data. If you have a scRNA-seq data which was processed with Seurat and saved as Rds file, you can use the following command to make anndata from Seurat object. The anndata object produced by this command can be used for input of celloracle.

```
seuratToAnndata YOUR_SEURAT_OBJECT.Rds OUTPUT_PATH
```

### 1.3.2 Python API

#### Custom class in celloracle

We define some custom classes in celloracle.

```
class celloracle.Links (name, links_dict={})  
    Bases: object
```

This is a class for the processing and visualization of GRNs. Links object stores cluster-specific GRNs and metadata. Please use “get\_links” function in Oracle object to generate Links object.

##### links\_dict

Dictionary that store unprocessed network data.

**Type** dictionary

##### filtered\_links

Dictionary that store filtered network data.

**Type** dictionary

##### merged\_score

Network scores.

**Type** pandas.dataframe

##### cluster

List of cluster name.

**Type** list of str

##### name

Name of clustering unit.

**Type** str

##### palette

DataFrame that store color information.

**Type** pandas.dataframe

```
filter_links (p=0.001,    weight='coef_abs',    thread_number=10000,    genelist_source=None,  
              genelist_target=None)
```

Filter network edges. In most cases, inferred GRN has non-significant random edges. We have to remove these edges before analyzing the network structure. You can do the filtering in any of the following ways.

- (1) Filter based on the p-value of the network edge. Please enter p-value for thresholding.
- (2) Filter based on network edge number. If you set the number, network edges will be filtered based on the order of a network score. The top n-th network edges with network weight will remain, and the other edges will be removed. The network data has several types of network weight, so you have to select which network weight do you want to use.
- (3) Filter based on an arbitrary gene list. You can set a gene list for source nodes or target nodes.

#### Parameters

- **p** (*float*) – threshold for p-value of the network edge.
- **weight** (*str*) – Please select network weight name for the filtering
- **genelist\_source** (*list of str*) – gene list to remain in regulatory gene nodes. Default is None.

- **genelist\_target** (*list of str*) – gene list to remain in target gene nodes. Default is None.

**get\_network\_entropy** (*value='coef\_abs'*)  
Calculate network entropy scores.

**Parameters** **value** (*str*) – Default is “coef\_abs”.

**get\_score** (*test\_mode=False*)

Get several network scores using R libraries. Make sure all dependent R libraries are installed in your environment before running this function. You can check the installation for the R libraries by running `test_installation()` in `network_analysis` module.

```
plot_cartography_scatter_per_cluster(gois=None, clusters=None, scatter=True,
                                         kde=False, auto_gene_annot=False, percentile=98,
                                         args_dot={'n_levels': 105}, args_line={'c': 'gray'}, args_annot={}, save=None)
```

Make a gene network cartography plot. Please read the original paper describing gene network cartography for more information. <https://www.nature.com/articles/nature03288>

#### Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **clusters** (*list of str*) – List of cluster name to analyze. If None, all clusters in `Links` object will be analyzed.
- **scatter** (*bool*) – Whether to make a scatter plot.
- **auto\_gene\_annot** (*bool*) – Whether to pick up genes to make an annotation.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 98.
- **args\_dot** (*dictionary*) – Arguments for scatter plot.
- **args\_line** (*dictionary*) – Arguments for lines in cartography plot.
- **args\_annot** (*dictionary*) – Arguments for annotation in plots.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_cartography\_term** (*goi, save=None*)

Plot the gene network cartography term like a heatmap. Please read the original paper of gene network cartography for the principle of gene network cartography. <https://www.nature.com/articles/nature03288>

#### Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_degree\_distributions** (*plot\_model=False, save=None*)

Plot the network degree distributions (the number of edge per gene). The network degree will be visualized in both linear scale and log scale.

#### Parameters

- **links** ([Links](#)) – See network\_analysis.Links class for detail.
- **plot\_model** (*bool*) – Whether to plot linear approximation line.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_network\_entropy\_distributions** (*update\_network\_entropy=False, save=None*)

Plot the distribution for network entropy. See the CellOracle paper for more detail.

#### Parameters

- **links** (*Links object*) – See network\_analysis.Links class for detail.
- **values** (*list of str*) – The list of score to visualize. If it is None, all network score (listed above) will be used.
- **update\_network\_entropy** (*bool*) – Whether to recalculate network entropy.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_score\_comparison\_2D** (*value, cluster1, cluster2, percentile=99, annot\_shifts=None, save=None*)

Make a scatter plot that compares specific network scores in two groups.

#### Parameters

- **links** ([Links](#)) – See network\_analysis.Links class for detail.
- **value** (*srt*) – The network score type.
- **cluster1** (*str*) – Cluster name. Network scores in cluster1 will be visualized in the x-axis.
- **cluster2** (*str*) – Cluster name. Network scores in cluster2 will be visualized in the y-axis.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 99.
- **annot\_shifts** (*(float, float)*) – Annotation visualization setting.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_score\_distributions** (*values=None, method='boxplot', save=None*)

Plot the distribution of network scores. An individual data point is a network edge (gene).

#### Parameters

- **links** ([Links](#)) – See Links class for details.
- **values** (*list of str*) – The list of score to visualize. If it is None, all of the network score will be used.
- **method** (*str*) – Plotting method. Select either “boxplot” or “barplot”.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_score\_per\_cluster** (*goi, save=None*)

Plot network score for a gene. This function visualizes the network score for a specific gene between clusters to get an insight into the dynamics of the gene.

#### Parameters

- **links** ([Links](#)) – See network\_analysis.Links class for detail.
- **goi** (*srt*) – Gene name.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_scores\_as\_rank** (*cluster*, *n\_gene*=50, *save*=None)

Pick up top n-th genes with high-network scores and make plots.

#### Parameters

- **links** ([Links](#)) – See network\_analysis.Links class for detail.
- **cluster** (*str*) – Cluster name to analyze.
- **n\_gene** (*int*) – Number of genes to plot. Default is 50.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**to\_hdf5** (*file\_path*)

Save object as hdf5.

**Parameters** **file\_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.links’

**class** `celloracle.Net` (*gene\_expression\_matrix*, *gem\_standerdized*=None, *TFinfo\_matrix*=None, *cell\_state*=None, *TFinfo\_dic*=None, *annotation*=None, *verbose*=True)

Bases: `object`

Net is a custom class for inferring sample-specific GRN from scRNA-seq data. This class is used inside the Oracle class for GRN inference. This class requires two types of information below.

- (1) Single-cell RNA-seq data: The Net class needs processed scRNA-seq data. Gene and cell filtering, quality check, normalization, log-transformation (but not scaling and centering) have to be done before starting the GRN calculation with this class. You can also use any arbitrary metadata (i.e., mRNA count, cell-cycle phase) for GRN input.
- (2) Potential regulatory connection (or base GRN): This method uses the list of potential regulatory TFs as input. This information can be calculated from ATAC-seq data using the motif-analysis module. If sample-specific ATAC-seq data is not available, you can use general TF-binding info derived from public ATAC-seq dataset of various tissue/cell type.

#### linkList

The results of the GRN inference.

**Type** `pandas.DataFrame`

#### all\_genes

An array of all genes that exist in the input gene expression matrix

**Type** `numpy.array`

#### embedding\_name

The key name name in `adata.obsm` containing dimensional reduction coordinates

**Type** `str`

#### annotation

Annotation. you can add custom annotation.

**Type** `dictionary`

#### coefs\_dict

Coefs of linear regression.

**Type** dictionary

**stats\_dict**  
Statistic values about coefs.

**Type** dictionary

**fitted\_genes**  
List of genes where the regression model was successfully calculated.

**Type** list of str

**failed\_genes**  
List of genes that were not assigned coefs

**Type** list of str

**cellstate**  
A metadata for GRN input

**Type** pandas.DataFrame

**TFinfo**  
Information about potential regulatory TFs.

**Type** pandas.DataFrame

**gem**  
Merged matrix made with gene\_expression\_matrix and cellstate matrix.

**Type** pandas.DataFrame

**gem\_standarized**  
Almost the same as gem, but the gene\_expression\_matrix was standarized.

**Type** pandas.DataFrame

**library\_last\_update\_date**  
Last update date of this code. This info is for code development. It can be deprecated in the future

**Type** str

**object\_initiation\_date**  
The date when this object was made.

**Type** str

**addAnnotation (annotation\_dictionary)**  
Add a new annotation.

**Parameters** **annotation\_dictionary** (*dictionary*) – e.g. {“sample\_name”: “NIH 3T3 cell”}

**addTFinfo\_dictionary (TFdict)**  
Add a new TF info to pre-existing TFdict.

**Parameters** **TFdict** (*dictionary*) – python dictionary of TF info.

**addTFinfo\_matrix (TFinfo\_matrix)**  
Load TF info dataframe.

**Parameters** **TFinfo** (*pandas.DataFrame*) – information about potential regulatory TFs.

**copy ()**  
Deepcopy itself

---

**fit\_All\_genes** (*bagging\_number*=200, *scaling*=True, *model\_method*='bagging\_ridge', *command\_line\_mode*=False, *log*=None, *alpha*=1, *verbose*=True)

Make ML models for all genes. The calculation will be performed in parallel using scikit-learn bagging function. You can select a modeling method (bagging\_ridge or bayesian\_ridge). This calculation usually takes a long time.

#### Parameters

- **bagging\_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether or not to scale regulatory gene expression values.
- **model\_method** (*str*) – ML model name. Please select either "bagging\_ridge" or "bayesian\_ridge"
- **command\_line\_mode** (*bool*) – Please select False if the calculation is performed on jupyter notebook.
- **log** (*logging object*) – log object to output log
- **alpha** (*int*) – Strength of regularization.
- **verbose** (*bool*) – Whether or not to show a progress bar.

**fit\_All\_genes\_parallel** (*bagging\_number*=200, *scaling*=True, *log*=None, *verbose*=10)

IMPORTANT: this function being debugged and is currently unavailable.

Make ML models for all genes. The calculation will be performed in parallel using joblib parallel module.

#### Parameters

- **bagging\_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether or not to scale regulatory gene expression values.
- **log** (*logging object*) – log object to output log
- **verbose** (*int*) – verbose for joblib parallel

**fit\_genes** (*target\_genes*, *bagging\_number*=200, *scaling*=True, *model\_method*='bagging\_ridge', *save\_coefs*=False, *command\_line\_mode*=False, *log*=None, *alpha*=1, *verbose*=True)

Make ML models for genes of interest. This calculation will be performed in parallel using scikit-learn's bagging function. You can select a modeling method; Please chose either bagging\_ridge or bayesian\_ridge.

#### Parameters

- **target\_genes** (*list of str*) – gene list
- **bagging\_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether or not to scale regulatory gene expression values.
- **model\_method** (*str*) – ML model name. Please select either "bagging\_ridge" or "bayesian\_ridge"
- **save\_coefs** (*bool*) – Whether or not to store details of coef values in bagging model.
- **command\_line\_mode** (*bool*) – Please select False if the calculation is performed on jupyter notebook.
- **log** (*logging object*) – log object to output log
- **alpha** (*int*) – Strength of regularization.
- **verbose** (*bool*) – Whether or not to show a progress bar.

**plotCoefs** (*target\_gene*, *sort*=True, *threshold\_p*=None)

Plot the distribution of Coef values (network edge weights).

## Parameters

- **target\_gene** (*str*) – gene name
- **sort** (*bool*) – Whether or not to sort genes by its strength
- **bagging\_number** (*int*) – The number of estimators for bagging.
- **threshold\_p** (*float*) – the threshold for p-values. TFs will be filtered based on the p-value. if None, no filtering is applied.

**to\_hdf5** (*file\_path*)

Save object as hdf5.

**Parameters** **file\_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.net’

**updateLinkList** (*verbose=True*)

Update LinkList. LinkList is a data frame that store information about inferred GRNs.

**Parameters** **verbose** (*bool*) – Whether or not to show a progress bar

**updateTFinfo\_dictionary** (*TFdict*)

Update TF info matrix

**Parameters** **TFdict** (*dictionary*) – A python dictionary in which a key is Target gene, value are potential regulatory genes for the target gene.

**class** `celloracle.Oracle`

Bases: `celloracle.trajectory.modified_VelocytoLoom_class.modified_VelocytoLoom`

Oracle is the main class in CellOracle. Oracle object imports scRNA-seq data (anndata) and TF information to infer cluster-specific GRNs. It can predict the future gene expression patterns and cell state transitions in response to the perturbation of TFs. Please see the CellOracle paper for details. The code of the Oracle class was made of the three components below.

- (1) Anndata: Gene expression matrix and metadata from single-cell RNA-seq are stored in the anndata object. Processed values, such as normalized counts and simulated values, are stored as layers of anndata. Metadata (i.e., Cluster info) are saved in anndata.obs. Refer to scanpy/anndata documentation for detail.
- (2) Net: Net is a custom class in celloracle. Net object processes several data to infer GRN. See the Net class documentation for details.
- (3) VelocytoLoom: Calculation of transition probability and visualization of directed trajectory graph will be performed in the same way as velocytoloom. VelocytoLoom is class from Velocyto, a python library for RNA-velocity analysis. In celloracle, we use some functions in velocytoloom for the visualization.

**adata**

Imported anndata object

**Type** anndata

**cluster\_column\_name**

The column name in adata.obs containing cluster info

**Type** str

**embedding\_name**

The key name in adata.obsm containing dimensional reduction coordinates

**Type** str

**addTFinfo\_dictionary** (*TFdict*)

Add new TF info to pre-existing TFdict. Values in the old TF dictionary will remain.

**Parameters** `TFdict` (*dictionary*) – Python dictionary of TF info.

**copy()**

Deepcopy itself.

**count\_cells\_in\_mc\_resutls** (*cluster\_use*, *end=-1*, *order=None*)

Count the simulated cell by the cluster.

**Parameters**

- **cluster\_use** (*str*) – cluster information name in anndata.obs. You can use any cluster information in anndata.obs.
- **end** (*int*) – The end point of Sankey-diagram. Please select a step in the Markov simulation. if you set [end=-1], the final step of Markov simulation will be used.

**Returns** Number of cells before / after simulation

**Return type** pandas.DataFrame

**fit\_GRN\_for\_simulation** (*GRN\_unit='cluster'*, *alpha=1*, *use\_cluster\_specific\_TFdict=False*)

Do GRN inference. Please see the paper of CellOracle paper for details.

GRN can be constructed for the entire population or each clusters. If you want to infer cluster-specific GRN, please set [GRN\_unit="cluster"]. You can select cluster information when you import data.

If you set [GRN\_unit="whole"], GRN will be made using all cells.

**Parameters**

- **GRN\_unit** (*str*) – Select “cluster” or “whole”
- **alpha** (*float or int*) – The strength of regularization. If you set a lower value, the sensitivity increases, and you can detect weaker network connections. However, there may be more noise. If you select a higher value, it will reduce the chance of overfitting.

**get\_cluster\_specific\_TFdict\_from\_Links** (*links\_object*)

Extract TF and its target gene information from Links object. This function can be used to reconstruct GRNs based on pre-existing GRNs saved in Links object.

**Parameters** `links_object` (`Links`) – Please see the explanation of Links class.

**get\_links** (*cluster\_name\_for\_GRN\_unit=None*, *alpha=10*, *bagging\_number=20*, *verbose\_level=1*, *test\_mode=False*)

Makes GRN for each cluster and returns results as a Links object. Several preprocessing should be done before using this function.

**Parameters**

- **cluster\_name\_for\_GRN\_unit** (*str*) – Cluster name for GRN calculation. The cluster information should be stored in Oracle.adata.obs.
- **alpha** (*float or int*) – The strength of regularization. If you set a lower value, the sensitivity increases, and you can detect weaker network connections. However, there may be more noise. If you select a higher value, it will reduce the chance of overfitting.
- **bagging\_number** (*int*) – The number used in bagging calculation.
- **verbose\_level** (*int*) – if [verbose\_level>1], most detailed progress information will be shown. if [verbose\_level > 0], one progress bar will be shown. if [verbose\_level == 0], no progress bar will be shown.
- **test\_mode** (*bool*) – If test\_mode is True, GRN calculation will be done for only one cluster rather than all clusters.

```
import_TF_data (TF_info_matrix=None, TF_info_matrix_path=None, TFdict=None)
```

Load data about potential-regulatory TFs. You can import either TF\_info\_matrix or TFdict. For more information on how to make these files, please see the motif analysis module within the celloracle tutorial.

#### Parameters

- **TF\_info\_matrix** (`pandas.DataFrame`) – TF\_info\_matrix.
- **TF\_info\_matrix\_path** (`str`) – File path for TF\_info\_matrix (`pandas.DataFrame`).
- **TFdict** (`dictionary`) – Python dictionary of TF info.

```
import_anndata_as_normalized_count (adata, cluster_column_name=None, embedding_name=None)
```

Load scRNA-seq data. scRNA-seq data should be prepared as an anndata object. Preprocessing (cell and gene filtering, dimensional reduction, clustering, etc.) should be done before loading data. The method will import NORMALIZED and LOG TRANSFORMED data but NOT SCALED and NOT CENTERED data. See the tutorial for more details on how to process scRNA-seq data.

#### Parameters

- **adata** (`anndata`) – anndata object containing scRNA-seq data.
- **cluster\_column\_name** (`str`) – the name of column containing cluster information in `anndata.obs`. Clustering data should be in `anndata.obs`.
- **embedding\_name** (`str`) – the key name for dimensional reduction information in `anndata.obsm`. Dimensional reduction (or 2D trajectory graph) should be in `anndata.obsm`.
- **transform** (`str`) – The method for log-transformation. Choose one from “natural\_log” or “log2”.

```
import_anndata_as_raw_count (adata, cluster_column_name=None, embedding_name=None, transform='natural_log')
```

Load scRNA-seq data. scRNA-seq data should be prepared as an anndata object. Preprocessing (cell and gene filtering, dimensional reduction, clustering, etc.) should be done before loading data. The method imports RAW GENE COUNTS because unscaled and uncentered gene expression data are required for the GRN inference and simulation. See tutorial notebook for the details about how to process scRNA-seq data.

#### Parameters

- **adata** (`anndata`) – anndata object that stores scRNA-seq data.
- **cluster\_column\_name** (`str`) – the name of column containing cluster information in `anndata.obs`. Clustering data should be in `anndata.obs`.
- **embedding\_name** (`str`) – the key name for dimensional reduction information in `anndata.obsm`. Dimensional reduction (or 2D trajectory graph) should be in `anndata.obsm`.
- **transform** (`str`) – The method for log-transformation. Choose one from “natural\_log” or “log2”.

```
plot_mc_result_as_kde (n_time, args={})
```

Pick up one timepoint in the cell state-transition simulation and plot as a kde plot.

#### Parameters

- **n\_time** (`int`) – the number in Markov simulation
- **args** (`dictionary`) – An argument for `seaborn.kdeplot`. See `seaborn` documentation for details (<https://seaborn.pydata.org/generated/seaborn.kdeplot.html#seaborn.kdeplot>).

**plot\_mc\_result\_as\_trajectory**(*cell\_name*, *time\_range*, *args*={})

Pick up several timepoints in the cell state-transition simulation and plot as a line plot. This function can be used to visualize how cell-state changes after perturbation focusing on a specific cell.

**Parameters**

- **cell\_name** (*str*) – cell name. chose from adata.obs.index
- **time\_range** (*list of int*) – the list of index in Markov simulation
- **args** (*dictionary*) – dictionary for the arguments for matplotlib.pyplot.plot. See matplotlib documentation for details ([https://matplotlib.org/api/\\_as\\_gen/matplotlib.pyplot.plot.html#matplotlib.pyplot.plot](https://matplotlib.org/api/_as_gen/matplotlib.pyplot.plot.html#matplotlib.pyplot.plot)).

**plot\_mc\_results\_as\_sankey**(*cluster\_use*, *start*=0, *end*=-1, *order*=None, *font\_size*=10)

Plot the simulated cell state-transition as a Sankey-diagram after groping by the cluster.

**Parameters**

- **cluster\_use** (*str*) – cluster information name in anndata.obs. You can use any cluster information in anndata.obs.
- **start** (*int*) – The starting point of Sankey-diagram. Please select a step in the Markov simulation.
- **end** (*int*) – The end point of Sankey-diagram. Please select a step in the Markov simulation. if you set [end=-1], the final step of Markov simulation will be used.
- **order** (*list of str*) – The order of cluster name in the Sankey-diagram.
- **font\_size** (*int*) – Font size for cluster name label in the Sankey diagram.

**prepare\_markov\_simulation**(*verbose*=False)

Pick up cells for Markov simulation.

**Parameters verbose** (*bool*) – If True, it plots selected cells.**run\_markov\_chain\_simulation**(*n\_steps*=500, *n\_duplication*=5, *seed*=123, *calculation\_randomized*=True)

Do Markov simulations to predict cell transition after perturbation. The transition probability between cells has been calculated based on simulated gene expression values in the signal propagation process. The cell state transition will be simulated based on the probability. You can simulate the process multiple times to get a robust outcome.

**Parameters**

- **n\_steps** (*int*) – steps for Markov simulation. This value is equivalent to the amount of time after perturbation.
- **n\_duplication** (*int*) – the number for multiple calculations.

**simulate\_shift**(*perturb\_condition*=None, *GRN\_unit*='cluster', *n\_propagation*=3, *ignore\_warning*=False)

Simulate signal propagation with GRNs. Please see the CellOracle paper for details. This function simulates a gene expression pattern in the near future. Simulated values will be stored in anndata.layers: [“simulated\_count”]

The simulation use three types of data. (1) GRN inference results (coef\_matrix). (2) Perturb\_condition: You can set arbitrary perturbation condition. (3) Gene expression matrix: The simulation starts from imputed gene expression data.

**Parameters**

- **perturb\_condition** (*dictionary*) – condition for perturbation. if you want to simulate knockout for GeneX, please set [perturb\_condition={“GeneX”: 0.0}] Although

you can set any non-negative values for the gene condition, avoid setting biologically infeasible values for the perturb condition. It is strongly recommended to check gene expression values in your data before selecting the perturb condition.

- **GRN\_unit** (*str*) – GRN type. Please select either “whole” or “cluster”. See the documentation of “fit\_GRN\_for\_simulation” for the detailed explanation.
- **n\_propagation** (*int*) – Calculation will be performed iteratively to simulate signal propagation in GRN. You can set the number of steps for this calculation. With a higher number, the results may recapitulate signal propagation for many genes. However, a higher number of propagation may cause more error/noise.

**summarize\_mc\_results\_by\_cluster** (*cluster\_use*, *random=False*)

This function summarizes the simulated cell state-transition by groping the results into each cluster. It returns summarized results as a pandas.DataFrame.

**Parameters** **cluster\_use** (*str*) – cluster information name in anndata.obs. You can use any arbitrary cluster information in anndata.obs.

**to\_hdf5** (*file\_path*)

Save object as hdf5.

**Parameters** **file\_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.oracle’

**updateTFinfo\_dictionary** (*TFdict*)

Update a TF dictionary. If a key in the new TF dictionary already exists in the old TF dictionary, old values will be replaced with a new one.

**Parameters** **TFdict** (*dictionary*) – Python dictionary of TF info.

**celloracle.load\_hdf5** (*file\_path*, *object\_class\_name=None*)

Load an object of celloracle’s custom class that was saved as hdf5.

**Parameters**

- **file\_path** (*str*) – file\_path.
- **object\_class\_name** (*str*) – Types of object. If it is None, object class will be identified from the extension of file\_name. Default is None.

## Modules for ATAC-seq analysis

### celloracle.motif\_analysis module

The *motif\_analysis* module implements transcription factor motif scan.

Genomic activity information (peak of ATAC-seq or Chip-seq) is extracted first. Then the peak DNA sequence will be subjected to TF motif scan. Finally we will get list of TFs that potentially binds to a specific gene.

**class** `celloracle.motif_analysis.TFinfo(peak_data_frame, ref_genome)`  
Bases: object

This is a custom class for motif analysis in celloracle. TFinfo object performs motif scan using the TF motif database in gimmemotifs and several functions of genomepy. Analysis results can be exported as a python dictionary or dataframe. These files; python dictionary of dataframe of TF binding information, are needed during GRN inference.

**peak\_df**

dataframe about DNA peak and target gene data.

**Type** pandas.dataframe

**all\_target\_gene**  
target genes.  
**Type** array of str

**ref\_genome**  
reference genome name that was used in DNA peak generation.  
**Type** str

**scanned\_df**  
Results of motif scan. Key is a peak name. Value is a dataframe of motif scan.  
**Type** dictionary

**dic\_targetgene2TFs**  
Final product of motif scan. Key is a target gene. Value is a list of regulatory candidate genes.  
**Type** dictionary

**dic\_peak2Targetgene**  
Dictionary. Key is a peak name. Value is a list of the target gene.  
**Type** dictionary

**dic\_TF2targetgenes**  
Final product of motif scan. Key is a TF. Value is a list of potential target genes of the TF.  
**Type** dictionary

**copy()**  
Deepcopy itself.

**filter\_motifs\_by\_score**(*threshold*, *method*=‘cumulative\_score’)  
Remove motifs with low binding scores.  
**Parameters** **method** (str) – thresholding method. Select either of [“individual\_score”, “cumulative\_score”]

**filter\_peaks**(*peaks\_to\_be\_remainded*)  
Filter peaks.  
**Parameters** **peaks\_to\_be\_remainded**(array of str) – list of peaks. Peaks that are NOT in the list will be removed.

**make\_TFinfo\_dataframe\_and\_dictionary**(*verbose*=True)  
This is the final step of motif\_analysis. Convert scanned results into a data frame and dictionaries.  
**Parameters** **verbose** (bool) – Whether to show a progress bar.

**reset\_dictionary\_and\_df()**  
Reset TF dictionary and TF dataframe. The following attributes will be erased: TF\_onehot, dic\_targetgene2TFs, dic\_peak2Targetgene, dic\_TF2targetgenes.

**reset\_filtering()**  
Reset filtering information. You can use this function to start over the filtering step with new conditions. The following attributes will be erased: TF\_onehot, dic\_targetgene2TFs, dic\_peak2Targetgene, dic\_TF2targetgenes.

**save\_as\_parquet**(*folder\_path*=None)  
Save itself. Some attributes are saved as parquet file.  
**Parameters** **folder\_path** (str) – folder path

**scan**(*background\_length*=200, *fpr*=0.02, *n\_cpus*=-1, *verbose*=True, *motifs*=None, *TF\_evidence\_level*=‘direct\_and\_indirect’)  
Scan DNA sequences searching for TF binding motifs.  
**Parameters**

- **background\_length** (*int*) – background length. This is used for the calculation of the binding score.
- **fpr** (*float*) – False positive rate for motif identification.
- **n\_cpus** (*int*) – number of CPUs for parallel calculation.
- **verbose** (*bool*) – Whether to show a progress bar.
- **motifs** (*list*) – a list of gimmemotifs motifs, will revert to default\_motifs() if None
- **TF\_evidence\_level** (*str*) – Please select one from [“direct”, “direct\_and\_indirect”]. If “direct” is selected, TFs that have a binding evidence were used. If “direct\_and\_indirect” is selected, TFs with binding evidence and inferred TFs are used. For more information, please read explanation of Motif class in gimmemotifs documentation (<https://gimmemotifs.readthedocs.io/en/master/index.html>)

**to\_dataframe** (*verbose=True*)

Return results as a dataframe. Rows are peak\_id, and columns are TFs.

**Parameters** **verbose** (*bool*) – Whether to show a progress bar.

**Returns** TFinfo matrix.

**Return type** pandas.dataframe

**to\_dictionary** (*dictionary\_type='targetgene2TFs'*, *verbose=True*)

Return TF information as a python dictionary.

**Parameters** **dictionary\_type** (*str*) – Type of dictionary. Select from [“targetgene2TFs”, “TF2targetgenes”]. If you chose “targetgene2TFs”, it returns a dictionary in which a key is a target gene, and a value is a list of regulatory candidate genes (TFs) of the target. If you chose “TF2targetgenes”, it returns a dictionary in which a key is a TF and a value is a list of potential target genes of the TF.

**Returns** dictionary.

**Return type** dictionary

**to\_hdf5** (*file\_path*)

Save object as hdf5.

**Parameters** **file\_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.tfinfo’

celloracle.motif\_analysis.**get\_tss\_info** (*peak\_str\_list*, *ref\_genome*, *verbose=True*)

Get annotation about Transcription Starting Site (TSS).

**Parameters**

- **peak\_str\_list** (*list of str*) – list of peak\_id. e.g.,  
[“chr5\_0930303\_9499409”, “chr11\_123445555\_123445577”]
- **ref\_genome** (*str*) – reference genome name.
- **verbose** (*bool*) – verbosity.

celloracle.motif\_analysis.**integrate\_tss\_peak\_with\_cicero** (*tss\_peak*, *cicero\_connections*)

Process output of cicero data and returns DNA peak information for motif analysis in celloracle. Please see the celloracle tutorial for more information.

**Parameters**

- **tss\_peak** (*pandas.DataFrame*) – dataframe about TSS information. Please use the function, “get\_tss\_info” to get this dataframe.
- **cicero\_connections** (*dataframe*) – dataframe that stores the results of cicero analysis.

**Returns** DNA peak about promoter/enhancer and its annotation about target gene.

**Return type** pandas.dataframe

`celloracle.motif_analysis.is_genome_installed(ref_genome)`

Celloracle motif\_analysis module uses gimmemotifs and genomepy internally. Reference genome files should be installed in the PC to use gimmemotifs and genomepy. This function checks the installation status of the reference genome.

**Parameters** `ref_genome` (`str`) – names of reference genome. i.e., “mm10”, “hg19”

`celloracle.motif_analysis.load_TFinfo(file_path)`

Load TFinfo object which was saved as hdf5 file.

**Parameters** `file_path` (`str`) – file path.

**Returns** Loaded TFinfo object.

**Return type** `TFinfo`

`celloracle.motif_analysis.load_TFinfo_from_parquets(folder_path)`

Load TFinfo object which was saved with the function; “save\_as\_parquet”.

**Parameters** `folder_path` (`str`) – folder path

**Returns** Loaded TFinfo object.

**Return type** `TFinfo`

`celloracle.motif_analysis.load_motifs(motifs_name)`

Load motifs from celloracle motif database

**Parameters** `motifs_name` (`str`) – Name of motifs.

**Returns** List of gimmemotifs.motif object.

**Return type** list

`celloracle.motif_analysis.make_TFinfo_from_scanned_file(path_to_raw_bed,`  
`path_to_scanned_result_bed,`  
`ref_genome)`

This function is currently an available.

`celloracle.motif_analysis.peak2fasta(peak_ids, ref_genome)`

Convert peak\_id into fasta object.

**Parameters**

- `peak_id` (`str or list of str`) – Peak\_id. e.g. “chr5\_0930303\_9499409” or it can be a list of peak\_id. e.g. “[“chr5\_0930303\_9499409”, “chr11\_123445555\_123445577”]”
- `ref_genome` (`str`) – Reference genome name. e.g. “mm9”, “mm10”, “hg19” etc

**Returns** DNA sequence in fasta format

**Return type** gimmemotifs.fasta object

`celloracle.motif_analysis.read_bed(bed_path)`

Load bed file and return as dataframe.

**Parameters** `bed_path` (`str`) – File path.

**Returns** bed file in dataframe.

**Return type** pandas.dataframe

```
celloracle.motif_analysis.remove_zero_seq(fasta_object)
    Remove DNA sequence with zero length

celloracle.motif_analysis.scan_dna_for_motifs(scanner_object,          motifs_object, sequence_object,
                                              verbose=True)
This is a wrapper function to scan DNA sequences searchig for Gene motifs.
```

**Parameters**

- **scanner\_object** (*gimmemotifs.scanner*) – Object that do motif scan.
- **motifs\_object** (*gimmemotifs.motifs*) – Object that stores motif data.
- **sequence\_object** (*gimmemotifs.fasta*) – Object that stores sequence data.

**Returns** scan results is stored in data frame.

**Return type** pandas.dataframe

## Modules for Network analysis

### celloracle.network\_analysis module

The *network\_analysis* module implements Network analysis.

```
class celloracle.network_analysis.Links(name, links_dict={})
```

Bases: object

This is a class for the processing and visualization of GRNs. Links object stores cluster-specific GRNs and metadata. Please use “get\_links” function in Oracle object to generate Links object.

**links\_dict**

Dictionary that store unprocessed network data.

**Type** dictionary

**filtered\_links**

Dictionary that store filtered network data.

**Type** dictionary

**merged\_score**

Network scores.

**Type** pandas.dataframe

**cluster**

List of cluster name.

**Type** list of str

**name**

Name of clustering unit.

**Type** str

**palette**

DataFrame that store color information.

**Type** pandas.dataframe

```
filter_links(p=0.001,           weight='coef_abs',           thread_number=10000,
```

```
            genelist_source=None, genelist_target=None)
```

Filter network edges. In most cases, inferred GRN has non-significant random edges. We have

to remove these edges before analyzing the network structure. You can do the filtering in any of the following ways.

- (1) Filter based on the p-value of the network edge. Please enter p-value for thresholding.
- (2) Filter based on network edge number. If you set the number, network edges will be filtered based on the order of a network score. The top n-th network edges with network weight will remain, and the other edges will be removed. The network data has several types of network weight, so you have to select which network weight do you want to use.
- (3) Filter based on an arbitrary gene list. You can set a gene list for source nodes or target nodes.

#### Parameters

- **p** (*float*) – threshold for p-value of the network edge.
- **weight** (*str*) – Please select network weight name for the filtering
- **genelist\_source** (*list of str*) – gene list to remain in regulatory gene nodes. Default is None.
- **genelist\_target** (*list of str*) – gene list to remain in target gene nodes. Default is None.

**get\_network\_entropy** (*value='coef\_abs'*)

Calculate network entropy scores.

**Parameters** **value** (*str*) – Default is “coef\_abs”.

**get\_score** (*test\_mode=False*)

Get several network scores using R libraries. Make sure all dependent R libraries are installed in your environment before running this function. You can check the installation for the R libraries by running `test_installation()` in `network_analysis` module.

```
plot_cartography_scatter_per_cluster(gois=None,           clusters=None,
                                      scatter=True,          kde=False,
                                      auto_gene_annot=False, percentile=98,
                                      args_dot={'n_levels': 105}, args_line={'c': 'gray'},
                                      args_annot={}, save=None)
```

Make a gene network cartography plot. Please read the original paper describing gene network cartography for more information. <https://www.nature.com/articles/nature03288>

#### Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **clusters** (*list of str*) – List of cluster name to analyze. If None, all clusters in `Links` object will be analyzed.
- **scatter** (*bool*) – Whether to make a scatter plot.
- **auto\_gene\_annot** (*bool*) – Whether to pick up genes to make an annotation.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 98.
- **args\_dot** (*dictionary*) – Arguments for scatter plot.
- **args\_line** (*dictionary*) – Arguments for lines in cartography plot.
- **args\_annot** (*dictionary*) – Arguments for annotation in plots.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_cartography\_term** (*goi, save=None*)

Plot the gene network cartography term like a heatmap. Please read the original paper of gene network cartography for the principle of gene network cartography. <https://www.nature.com/articles/nature03288>

#### Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.

- **gois** (*list of str*) – List of gene name to highlight.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_degree\_distributions** (*plot\_model=False, save=None*)

Plot the network degree distributions (the number of edge per gene). The network degree will be visualized in both linear scale and log scale.

**Parameters**

- **links** (*Links*) – See network\_analysis.Links class for detail.
- **plot\_model** (*bool*) – Whether to plot linear approximation line.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_network\_entropy\_distributions** (*update\_network\_entropy=False, save=None*)

Plot the distribution for network entropy. See the CellOracle paper for more detail.

**Parameters**

- **links** (*Links object*) – See network\_analysis.Links class for detail.
- **values** (*list of str*) – The list of score to visualize. If it is None, all network score (listed above) will be used.
- **update\_network\_entropy** (*bool*) – Whether to recalculate network entropy.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_score\_comparison\_2D** (*value, cluster1, cluster2, percentile=99, annot\_shifts=None, save=None*)

Make a scatter plot that compares specific network scores in two groups.

**Parameters**

- **links** (*Links*) – See network\_analysis.Links class for detail.
- **value** (*srt*) – The network score type.
- **cluster1** (*str*) – Cluster name. Network scores in cluster1 will be visualized in the x-axis.
- **cluster2** (*str*) – Cluster name. Network scores in cluster2 will be visualized in the y-axis.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 99.
- **annot\_shifts** (*(float, float)*) – Annotation visualization setting.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_score\_distributions** (*values=None, method='boxplot', save=None*)

Plot the distribution of network scores. An individual data point is a network edge (gene).

**Parameters**

- **links** (*Links*) – See Links class for details.
- **values** (*list of str*) – The list of score to visualize. If it is None, all of the network score will be used.
- **method** (*str*) – Plotting method. Select either “boxplot” or “barplot”.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_score\_per\_cluster** (*goi, save=None*)

Plot network score for a gene. This function visualizes the network score for a specific gene between clusters to get an insight into the dynamics of the gene.

#### Parameters

- **links** ([Links](#)) – See network\_analysis.Links class for detail.
- **goi** (*srt*) – Gene name.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**plot\_scores\_as\_rank** (*cluster, n\_gene=50, save=None*)

Pick up top n-th genes with high-network scores and make plots.

#### Parameters

- **links** ([Links](#)) – See network\_analysis.Links class for detail.
- **cluster** (*str*) – Cluster name to analyze.
- **n\_gene** (*int*) – Number of genes to plot. Default is 50.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

**to\_hdf5** (*file\_path*)

Save object as hdf5.

**Parameters** **file\_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.links’

`celloracle.network_analysis.draw_network(linkList, return_graph=False)`

Plot network graph.

#### Parameters

- **linkList** ([pandas.DataFrame](#)) – GRN saved as linkList.
- **return\_graph** (*bool*) – Whether to return graph object.

**Returns** Network X graph object.

**Return type** Graph object

`celloracle.network_analysis.get_R_path()`

`celloracle.network_analysis.get_links(oracle_object, cluster_name_for_GRN_unit=None, alpha=10, bagging_number=20, verbose_level=1, test_mode=False)`

Make GRN for each cluster and returns results as a Links object. Several preprocessing should be done before using this function.

#### Parameters

- **oracle\_object** ([Oracle](#)) – See Oracle module for detail.
- **cluster\_name\_for\_GRN\_unit** (*str*) – Cluster name for GRN calculation. The cluster information should be stored in Oracle.adata.obs.
- **alpha** (*float or int*) – The strength of regularization. If you set a lower value, the sensitivity increases, and you can detect weaker network connections. However, there may be more noise. If you select a higher value, it will reduce the chance of overfitting.
- **bagging\_number** (*int*) – The number used in bagging calculation.

- **verbose\_level** (*int*) – if [verbose\_level>1], most detailed progress information will be shown. if [verbose\_level > 0], one progress bar will be shown. if [verbose\_level == 0], no progress bar will be shown.
- **test\_mode** (*bool*) – If test\_mode is True, GRN calculation will be done for only one cluster rather than all clusters.

`celloracle.network_analysis.linkList_to_networkgraph(filteredlinkList)`  
Convert linkList into Graph object in NetworkX.

**Parameters** `filteredlinkList` (*pandas.DataFrame*) – GRN saved as linkList.

**Returns** Network X graph objenct.

**Return type** Graph object

`celloracle.network_analysis.load_links(file_path)`  
Load links object saved as a hdf5 file.

**Parameters** `file_path` (*str*) – file path.

**Returns** loaded links object.

**Return type** `Links`

`celloracle.network_analysis.set_R_path(R_path)`

`celloracle.network_analysis.test_R_libraries_installation(show_all_stdout=False)`  
CellOracle.network\_analysis use several R libraries for network analysis. This is a test function to check for instalation of the necessary R libraries.

`celloracle.network_analysis.transfer_scores_from_links_to_adata(adata,  
links,  
method='median')`

Transfer the summary of network scores (median or mean) per group from Links object into adata.

**Parameters**

- **adata** (*anndata*) – anndata
- **links** (`Links`) – Likns object
- **method** (*str*) – The method to summarize data.

## Other modules

### `celloracle.go_analysis module`

The `go_analysis` module implements Gene Ontology analysis. This module use goatools internally.

`celloracle.go_analysis.geneID2Symbol(IDs, species='mouse')`  
Convert Entrez gene id into gene symbol.

**Parameters**

- **IDs** (*array of str*) – Entrez gene id.
- **species** (*str*) – Select species. Either “mouse” or “human”.

**Returns** Gene symbol

**Return type** list of str

`celloracle.go_analysis.geneSymbol2ID(symbols, species='mouse')`  
Convert gene symbol into Entrez gene id.

**Parameters**

- **symbols** (*array of str*) – gene symbol
  - **species** (*str*) – Select species. Either “mouse” or “human”
- Returns** Entrez gene id  
**Return type** list of str

`celloracle.go_analysis.get_GO(gene_query, species='mouse')`

Get Gene Ontologies (GOs).

#### Parameters

- **gene\_query** (*array of str*) – gene list.
- **species** (*str*) – Select species. Either “mouse” or “human”

**Returns** GO analysis results as dataframe.

**Return type** pandas.dataframe

## celloracle.utility module

The `utility` module has several functions that support celloracle.

`celloracle.utility.exec_process(commands, message=True, wait_finished=True, turn_process=True)`

Excute a command. This is a wrapper of “`subprocess.Popen`”

#### Parameters

- **commands** (*str*) – command.
- **message** (*bool*) – Whether to return a message or not.
- **wait\_finished** (*bool*) – Whether or not to wait for the process to finish. If false, the process will be perfomed in background and the function will finish immediately
- **return\_process** (*bool*) – Whether to return “process”.

`celloracle.utility.intersect(list1, list2)`

Intersect two list and get components that exists in both list.

#### Parameters

- **list1** (*list*) – input list.
- **list2** (*list*) – input list.

**Returns** intersected list.

**Return type** list

`celloracle.utility.knn_data_transferer(adata_ref, adata_que, n_neighbors=20, cluster_name=None, embedding_name=None, adata_true=None, transfer_color=True, n_PCA=30, use_PCA_in_adata=False)`

Extract categorical information from `adata.obs` or embedding information from `adata.obsm` and transfer these information into query `anndata`. In the calculation, KNN is used after PCA.

#### Parameters

- **adata\_ref** (*anndata*) – reference `anndata`
- **adata\_que** (*anndata*) – query `anndata`
- **cluster\_name** (*str or list of str*) – cluster name(s) to be transferred. If you want to transfer multiple data, you can set the cluster names as a list.
- **embedding\_name** (*str or list of str*) – embedding name(s) to be transferred. If you want to transfer multiple data, you can set the embed-

- ding names as a list.
- **adata\_true** (*str*) – This argument can be used for the validation of this algorithm. If you have true answer, you can set it. If you set true answer, the function will return some metrics for benchmarking.
  - **transfer\_color** (*bool*) – Whether or not to transfer color data in addition to cluster information.
  - **n\_PCA** (*int*) – Number of PCs that will be used for the input of KNN algorithm.

```
celloracle.utility.load_hdf5(file_path, object_class_name=None)
```

Load an object of celloracle's custom class that was saved as hdf5.

**Parameters**

- **file\_path** (*str*) – file\_path.
- **object\_class\_name** (*str*) – Types of object. If it is None, object class will be identified from the extension of file\_name. Default is None.

```
celloracle.utility.load_pickled_object(filepath)
```

Load pickled object.

**Parameters** **filepath** (*str*) – file path.

**Returns** loaded object.

**Return type** python object

```
class celloracle.utility.makelog(file_name=None, directory=None)
```

Bases: object

This is a class for making log.

**info** (*comment*)

Add comment into the log file.

**Parameters** **comment** (*str*) – comment.

```
celloracle.utility.save_as_pickled_object(obj,filepath)
```

Save any object using pickle.

**Parameters**

- **obj** (*any python object*) – python object.
- **filepath** (*str*) – file path.

```
celloracle.utility.standard(df)
```

Standardize value.

**Parameters** **df** (*pandas.DataFrame*) – dataframe.

**Returns** Data after standardization.

**Return type** pandas.DataFrame

```
celloracle.utility.transfer_all_colors_between_anndata(adata_ref,  
                                                    adata_que)
```

Extract all color information from reference anndata and transfer the color into query anndata.

**Parameters**

- **adata\_ref** (*anndata*) – reference anndata
- **adata\_que** (*anndata*) – query anndata

```
celloracle.utility.transfer_color_between_anndata(adata_ref,  
                                                adata_que,  
                                                clus-  
                                                ter_name)
```

Extract color information from reference anndata and transfer the color into query anndata.

**Parameters**

- **adata\_ref** (*anndata*) – reference anndata
- **adata\_que** (*anndata*) – query anndata

- **cluster\_name** (*str*) – cluster name. This information should exist in the anndata.obs.

```
celloracle.utility.update_adata(adata)
```

## celloracle.data module

The `data` module implements data download and loading.

```
celloracle.data.load_TFinfo_df_mm9_mouse_atac_atlas()
```

Load Transcription factor binding information made from mouse scATAC-seq atlas dataset. mm9 genome was used for the reference genome.

Args:

**Returns** TF binding info.

**Return type** pandas.DataFrame

## celloracle.data\_conversion module

The `data_conversion` module implements data conversion between different platform.

```
celloracle.data_conversion.seurat_object_to_anndata(file_path_seurat_object,
                                                    delete_tmp_file=True)
```

Convert seurat object into anndata.

### Parameters

- **file\_path\_seurat\_object** (*str*) – File path of seurat object. Seurat object should be saved as Rds format.
- **delete\_tmp\_file** (*bool*) – Whether to delete temporary file.

**Returns** anndata object.

**Return type** anndata

## 1.4 Changelog

- **0.5.0 <2020-08-3>**
  - Add now functions for custom motifs. You can select motifs from several options. Also, we updated our web tutorial to introduce how to load / make a different motif data.
  - Change default motifs for S.cerevisiae and Zebrafish.
  - Change requirements for dependent package: gimmemotifs and geomepy. Celloracle codes were updated to support new version of gimmemotifs (0.14.4) and geomepy (0.8.4).
- **0.4.2 <2020-07-14>**
  - Add promoter-TSS information for Zebrafish reference genome (danRer7, danRer10 and danRer11).
- **0.4.1 <2020-07-02>**
  - Add promoter-TSS information for S.cerevisiae reference genome (sacCer2 and sacCer3).
- **0.4.0 <2020-06-28>**
  - Change requirements.
  - From this version, pandas version 1.0.3 or later is required.
  - From this version, scanpy version 1.5.3 or later is required.

- 0.3.7 <2020-06-12>
  - Delete GO function from r-script
  - Update some functions for network visualization
- 0.3.6 <2020-06-08>
  - Fix a bug on the transition probability calculation in Markov simulation
  - Add new function “count\_cells\_in\_mc\_results” to oracle class
- 0.3.5 <2020-05-09>
  - Fix a bug on the function for gene cortography visualization
  - Change some settings for installation
  - Update data conversion module
- 0.3.4 <2020-04-29>
  - Change pandas version restriction
  - Fix a bug on the function for gene cortography visualization
  - Add new functions for R-path configuration
- 0.3.3 <2020-04-24>
  - Add promoter-TSS information for hg19 and hg38 reference genome
- 0.3.1 <2020-03-23>
  - Fix an error when try to save file larger than 4GB file
- 0.3.0 <2020-2-17>
  - Release beta version

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## 1.6 Authors and citations

### 1.6.1 Cite celloracle

If you use celloracle please cite our bioarxiv preprint [CellOracle: Dissecting cell identity via network inference and in silico gene perturbation](#).

### 1.6.2 celloracle software development

celloracle is developed and maintained by [Kenji Kamimoto](#) and members of [Samantha Morris Lab](#). Please post troubles or questions on the [Github repository](#).



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TWO**

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