# celloracle

***Release 0.5.1***

**Samantha Morris Lab**

**Aug 04, 2020**

# CONTENTS

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

---

**Note:**

Documentation is also available as a pdf file.

`pdf documentation`

---

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

CHAPTER

ONE

# 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. Comming 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. Higer 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 llvm␣
↪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 (>=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 adavance.

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

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)
```

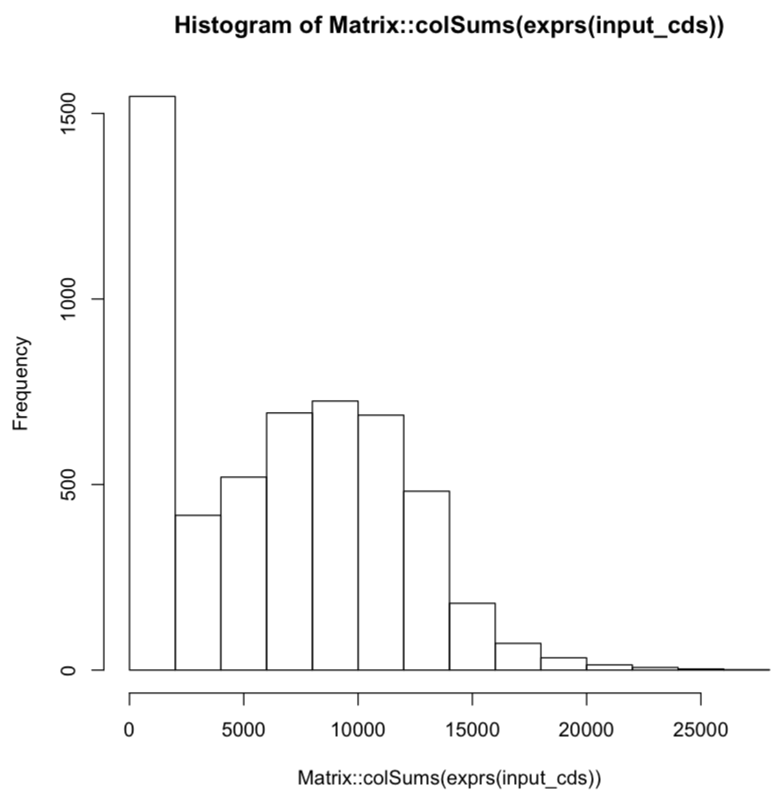
### 2. Load data and make Cell Data Set (CDS) object

#### 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. Qaulity check and Filtering

[8]:
```
# Visualize peak_count_per_cell
hist(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")
```

(continues on next page)

(continued from previous page)

```
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

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

[ ]:
```
# !!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]:
```
# runc 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"
```

(continues on next page)

(continued from previous page)

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

A data.frame: 6 × 3

| | Peak1 <fct> | Peak2 <fct> | coaccess <dbl> |
|---|---|---|---|
| 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 genoms.

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 | Slc25a26 | + |
| 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. Rerefence 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_foamat(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)
```

(continues on next page)

(continued from previous page)

```
    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_foamat(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 gimmemotifs. We have several option for loading motif database as below.

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

Many motif databases are included with GimmeMotifs. https://gimmemotifs.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 gimmemotifs.motif import MotifConfig
config = MotifConfig()
motif_dir = config.get_motif_dir()
```

(continues on next page)

(continued from previous page)

```
# 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 gimmemotifs.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_mCAwAwATrGwAAn,
 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()
```

[16]:
```
                     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.

## 4. 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.
```

(continues on next page)

(continued from previous page)

```
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='')))
```

## 5. Get Final results

### 5.1. Get resutls as a dictionary

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

### 5.2. 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]
```

#### 6. 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 dafault 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_snnGGsssGGs,
 GM.5.0.Nuclear_receptor.0001_TAwsTrGGTCAsTrGGTCA,
 GM.5.0.Mixed.0002_GCTAATTA,
 GM.5.0.Nuclear_receptor.0002_wnyrCTTCCGGGkC,
 GM.5.0.bHLH.0001_ACGTG,
 GM.5.0.Myb_SANT.0001_rrCCGTTAAACnGyy,
 GM.5.0.C2H2_ZF.0001_GCGkGGGCGG,
 GM.5.0.GATA.0001_TTATCTsnnnnnnnCA]
```

### 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
```

(continues on next page)

(continued from previous page)

```
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_mCAwAwATrGwAAn,
 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_nrAACAATAnn,
 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_nnnGCCCCnn,
 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 thrid 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
```

(continues on next page)

(continued from previous page)

```
# 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]:
```python
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_mCAwAwATrGwAAn,
 MA0096.1_bZIP910_mTGACGT]
```

In another notebook, we introduce how to make XXX.pfm file and XXX.motif2factors.txt file. Pleas 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 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.motif2facrors.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 dadabase

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’
```

(continues on next page)

(continued from previous page)

```
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

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

from datetime import datetime
import glob

def read_pwn_and_convert_into_list(path):
    # read pwn 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.ccbr.utoronto.ca/data/2.00/DataFiles/
↪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)]
```

(continues on next page)

(continued from previous page)

```
    # 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 gimmemotif 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_castellanii
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_macrogynus
Alternaria_brassicicola
Amanita_muscaria
Amborella_trichopoda
Amphimedon_queenslandica
Anas_platyrhynchos
```

(continues on next page)

(continued from previous page)

```
Ancylostoma_caninum
Ancylostoma_ceylanicum
Ancylostoma_duodenale
Angiostrongylus_cantonensis
Angiostrongylus_costaricensis
Anisakis_simplex
Anncaliia_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
```

(continues on next page)

(continued from previous page)

```
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
```

(continues on next page)

(continued from previous page)

```
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_immunda
Cladophialophora_psammophila
Cladophialophora_yegresii
Claviceps_purpurea
Clavispora_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
```

(continues on next page)

(continued from previous page)

```
Cupiennius_salei
Cyanidioschyzon_merolae
Cylicostephanus_goldi
Cyphellophora_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
```

(continues on next page)

(continued from previous page)

```
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
Gymnopus_luxurians
Haemonchus_contortus
Haemonchus_placei
Halocynthia_roretzi
Halyomorpha_halys
Harpegnathos_saltator
Hebeloma_cylindrosporum
Helianthus_annuus
Heliconius_melpomene
Heligmosomoides_bakeri
Helobdella_robusta
Hemiselmis_andersenii
Heterobasidion_annosum
```

(continues on next page)

(continued from previous page)

```
Heterobasidion_irregulare
Heterodontus_francisci
Heterorhabditis_bacteriophora
Histoplasma_capsulatum
Homo_sapiens
Hordeum_vulgare
Hyaloperonospora_arabidopsidis
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
Macrophomina_phaseolina
Macropus_eugenii
Magnaporthe_oryzae
Magnaporthe_poae
Malassezia_globosa
```

(continues on next page)

(continued from previous page)

```
Malassezia_sympodialis
Malus_domestica
Manihot_esculenta
Marmota_monax
Marssonina_brunnea
Medicago_sativa
Medicago_truncatula
Megaselia_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_haematococca
Nematocida_parisii_ertm1
Nematocida_sp
Nematostella_vectensis
```

(continues on next page)

(continued from previous page)

```
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
```

(continues on next page)

(continued from previous page)

```
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
```

(continues on next page)

(continued from previous page)

```
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
Saccharomycetaceae_sp_ashbya_aceri
Saccoglossus_kowalevskii
Salix_purpurea
Salpingoeca_rosetta
```

(continues on next page)

(continued from previous page)

```
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
```

(continues on next page)

(continued from previous page)

```
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_blattae
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
```

(continues on next page)

(continued from previous page)

```
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_alfalfae
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 gimmemotif 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 gimmemotifs.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_nrAACAATAnn,
 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 cont 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 neihbors 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

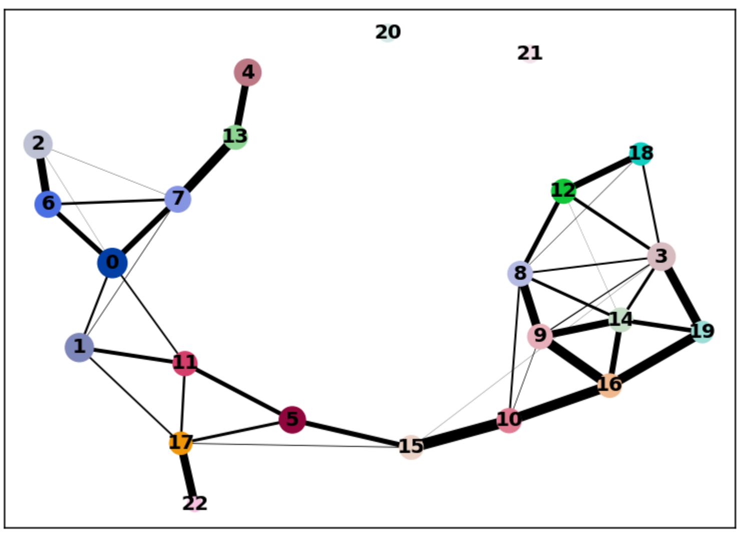
[12]:
```
# PAGA graph construction
sc.tl.paga(adata, groups='louvain')
```

[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]
```

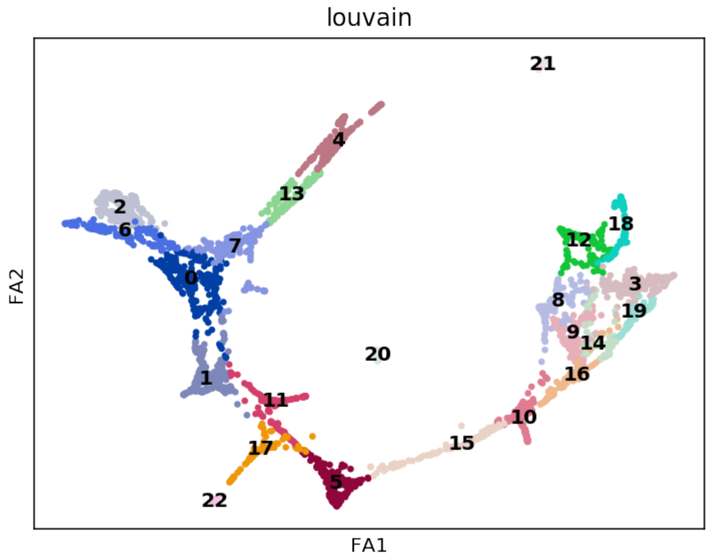
[14]:
```
plt.rcParams["figure.figsize"] = [6, 4.5]
```

[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')
```



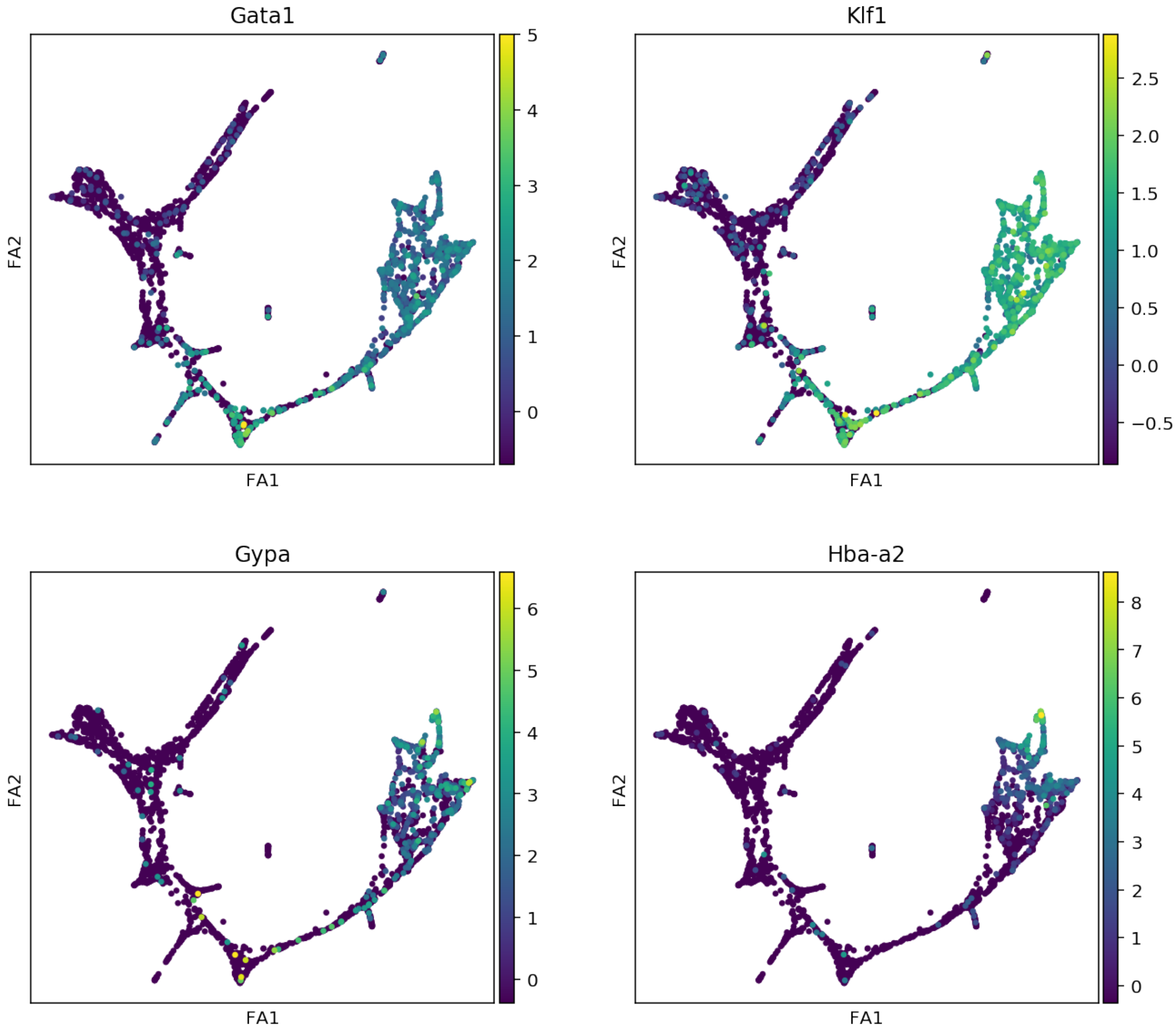

### 8. Check data

#### 8.1. Visualize marker gene expression

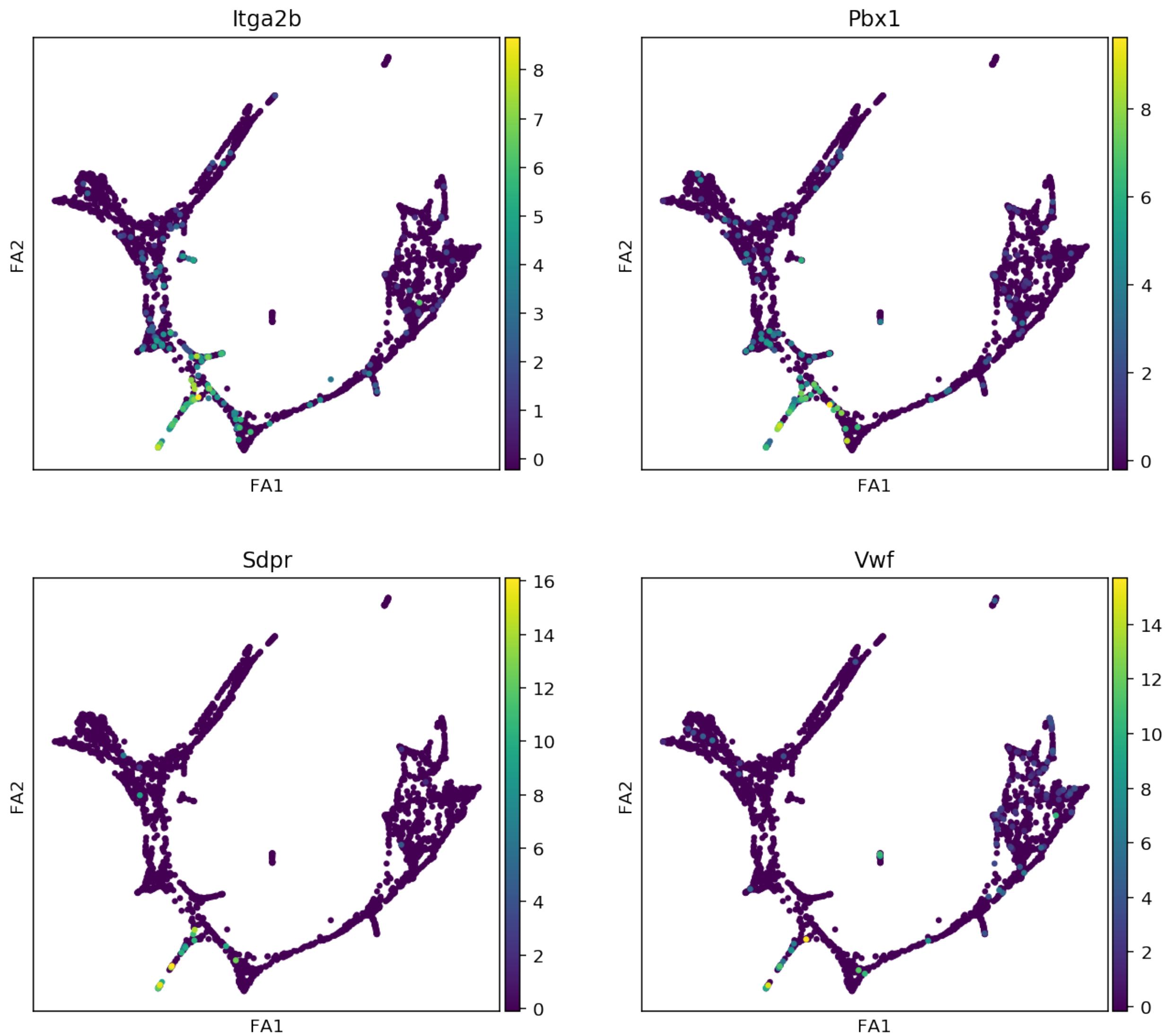
[18]:
```
plt.rcParams["figure.figsize"] = [4.5, 4.5]
```

[19]:
```
markers = {"Erythroids":["Gata1", "Klf1", "Gypa", "Hba-a2"],
           "Megakaryocytes":["Itga2b", "Pbx1", "Sdpr", "Vwf"],
            "Granulocytes":["Elane", "Cebpe", "Ctsg", "Mpo", "Gfi1"],
            "Monocytes":["Irf8", "Csf1r", "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()
```

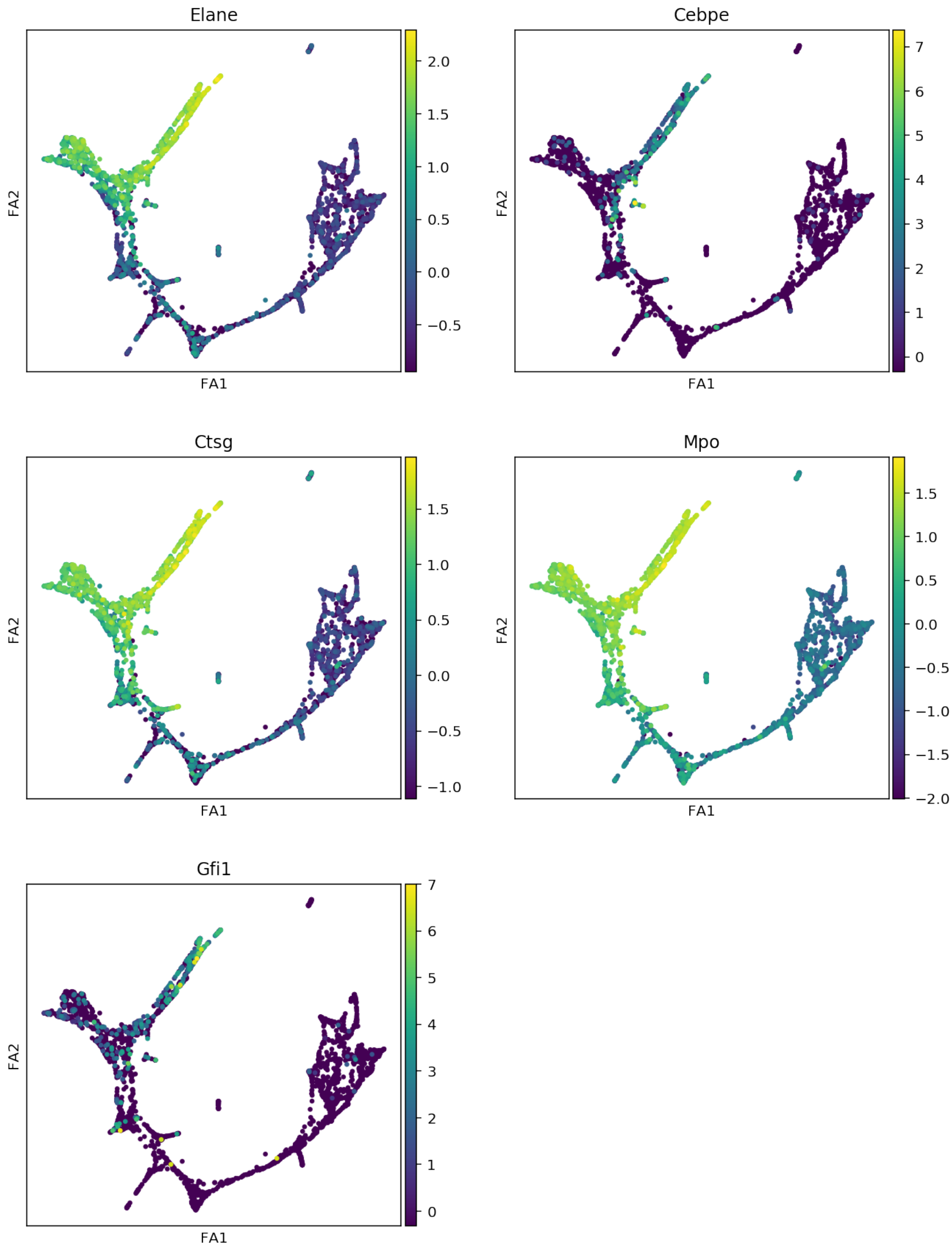
```
marker gene of Erythroids
```



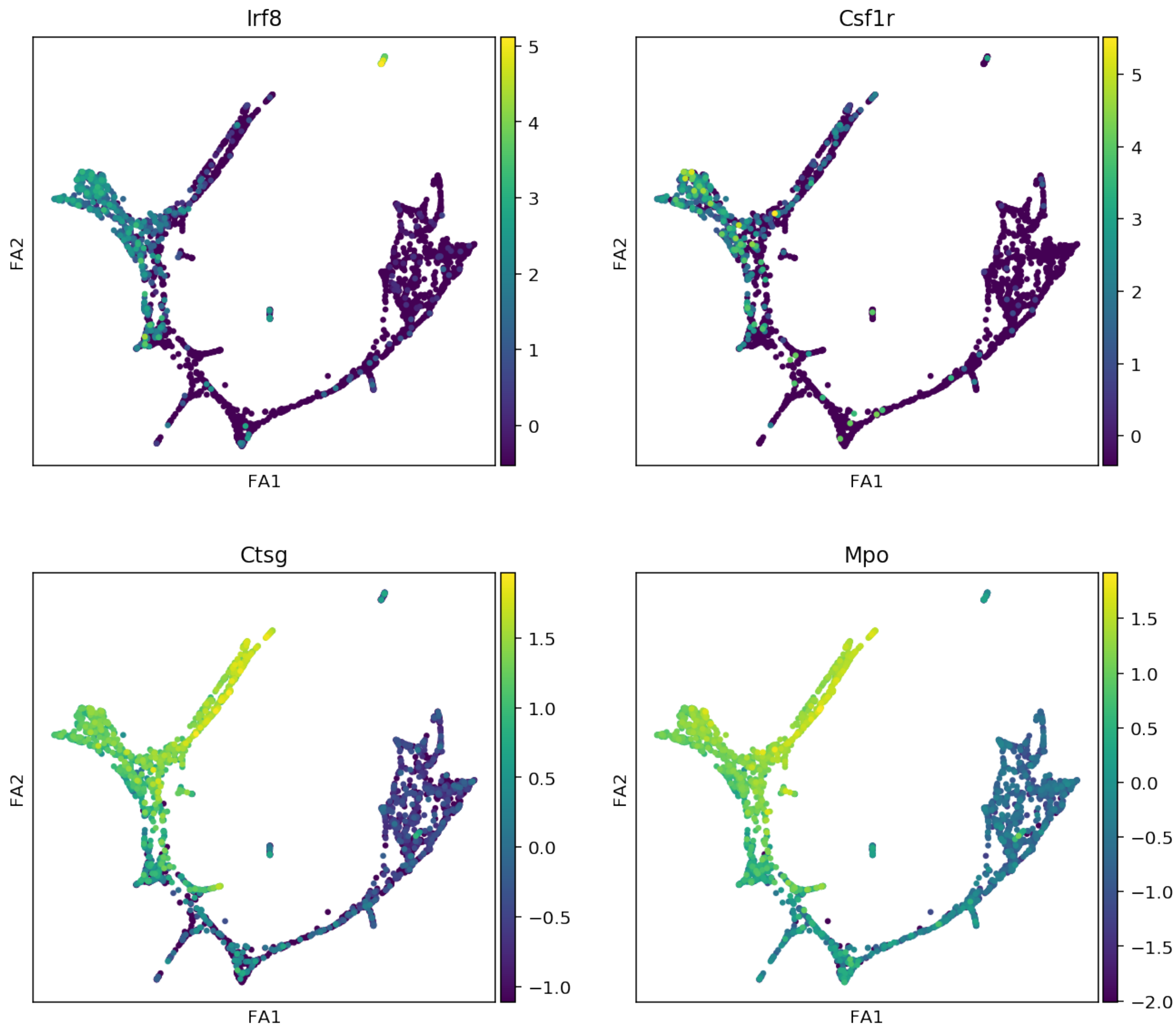

```
marker gene of Megakaryocytes
```



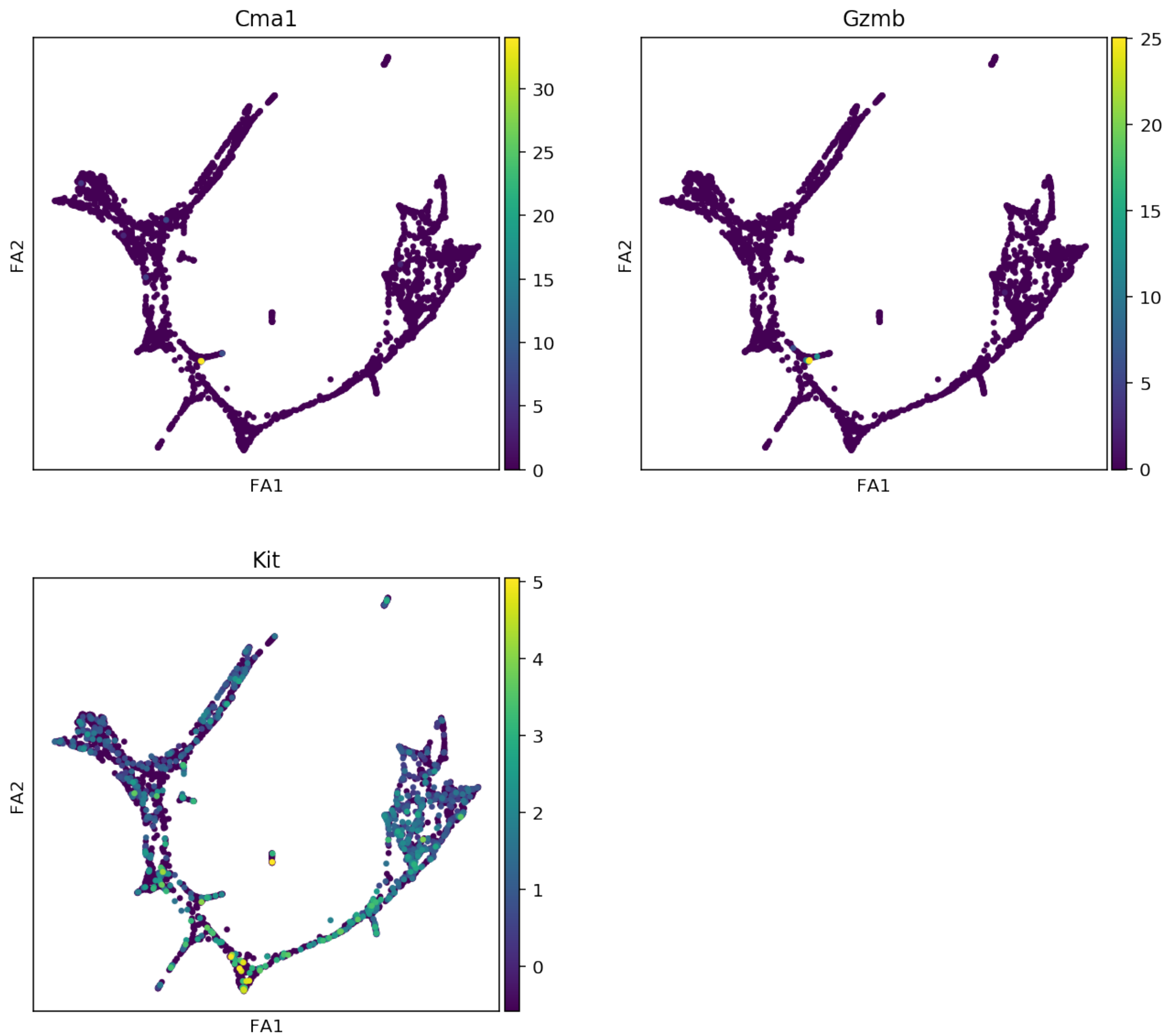

```
marker gene of Granulocytes
```



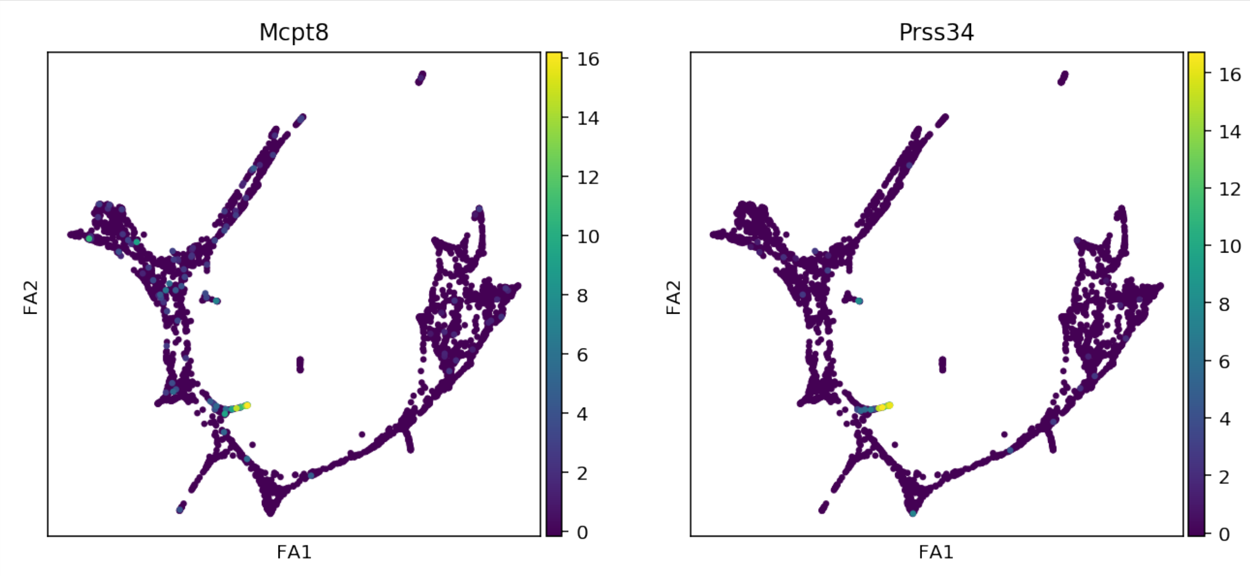

```
marker gene of Monocytes
```




```
marker gene of Mast_cells
```




```
marker gene of Basophils
```

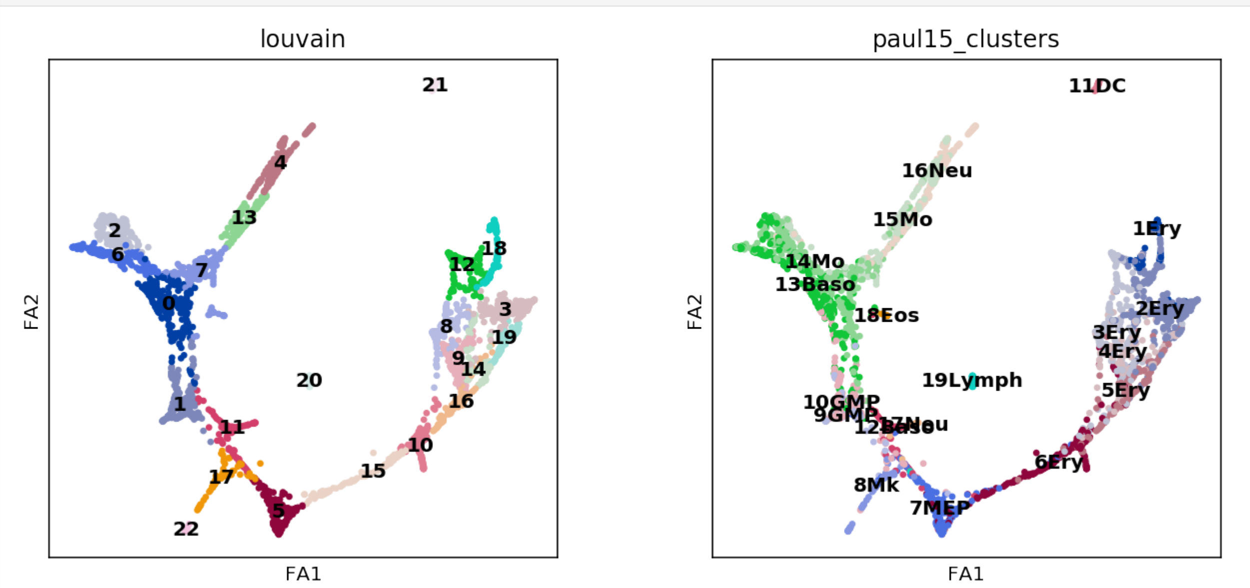



### 9. Make annotation for cluster

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.**

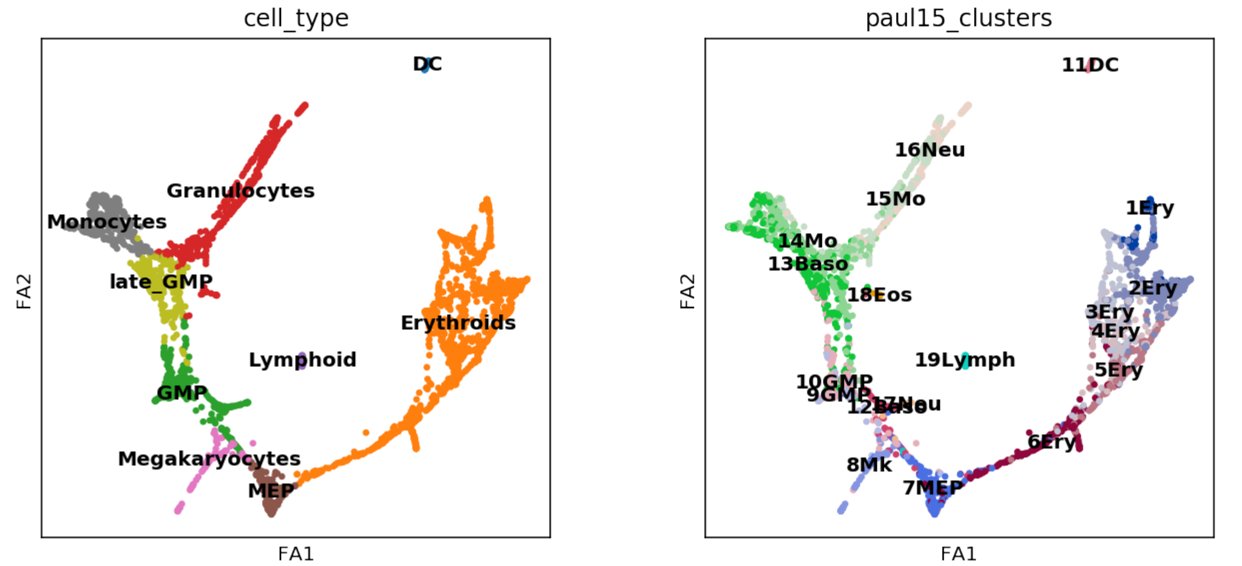
[22]:
```
# Make anottation dictionary
annotation = {"MEP":[5],
              "Erythroids": [15, 10, 16, 9, 8, 14, 19, 3, 12, 18],
              "Megakaryocytes":[17, 22],
              "GMP":[11, 1],
              "late_GMP" :[0],
              "Granulocytes":[7, 13, 4],
              "Monocytes":[6,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

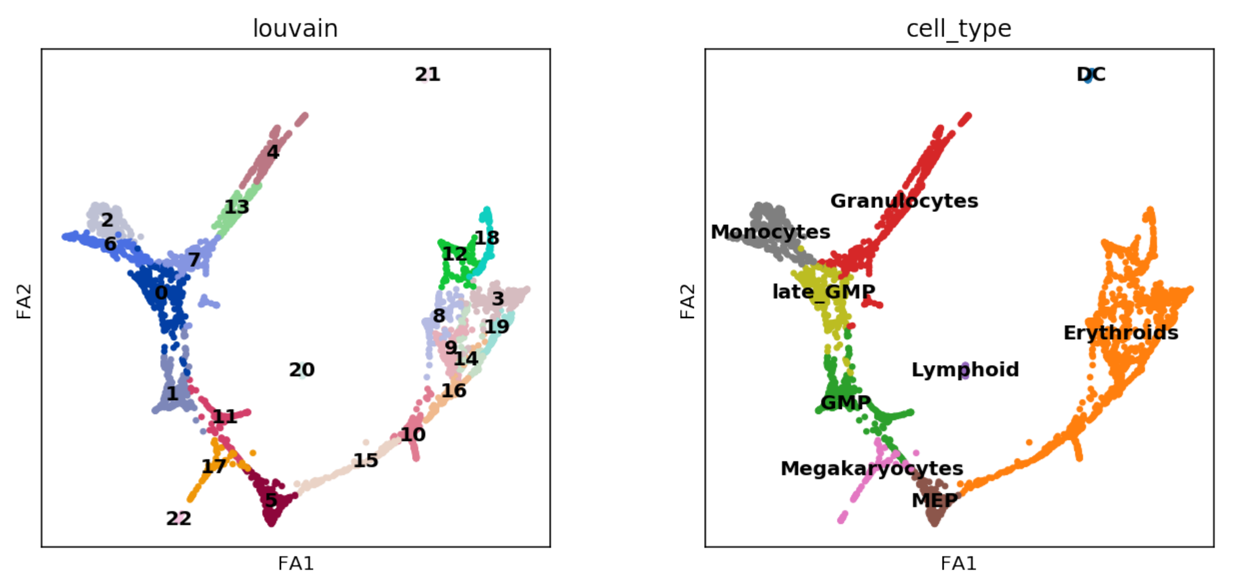
```
... storing 'cell_type' as categorical
```




## 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')
```



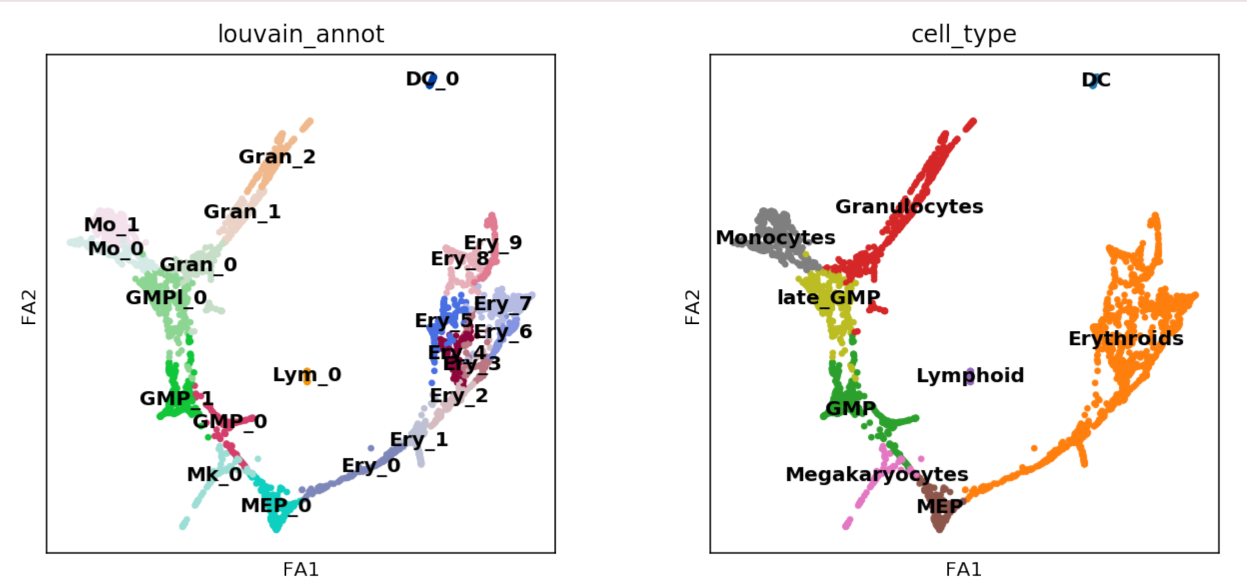
**!! Please change the dictionary below depending on the clustering results. The results may change depending on the execution environment.**

[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': 'GMPl_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 secveral 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

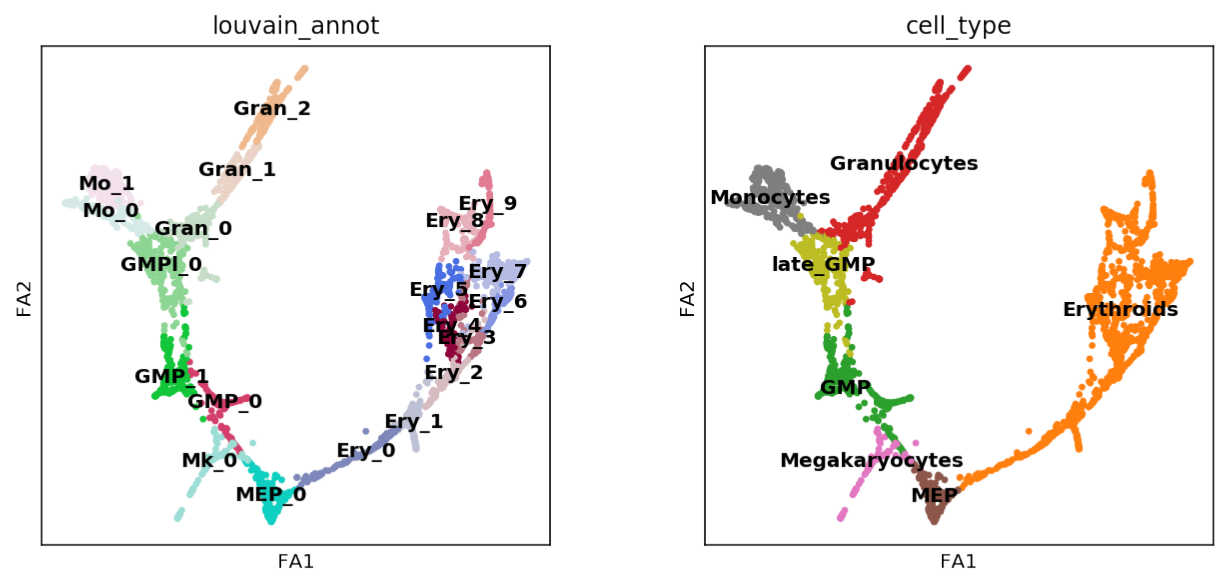
In this tutorial, we are using scRNA-seq data of hematopoiesis. In the latter part, we will focus on the cell fate decision in the myeloid lineage. So we will remove non-myeloid cell cluster; DC and Lymphoid cell cluster.

```
[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_etal_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_etal_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()
```

[11]:
```
                      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
1         0.0         0.0  0.0  0.0   0.0   0.0  ...     0.0   0.0     0.0
```

(continues on next page)

(continued from previous page)

```
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

   Znf85  Zscan10  Zscan16  Zscan22  Zscan26  Zscan31  Zscan4
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      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
4    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 to 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 informmation 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
```

[50]:
```
      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  Sfpi1  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 informatio dictionary into the oracle object

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

## 3. Knn imputation

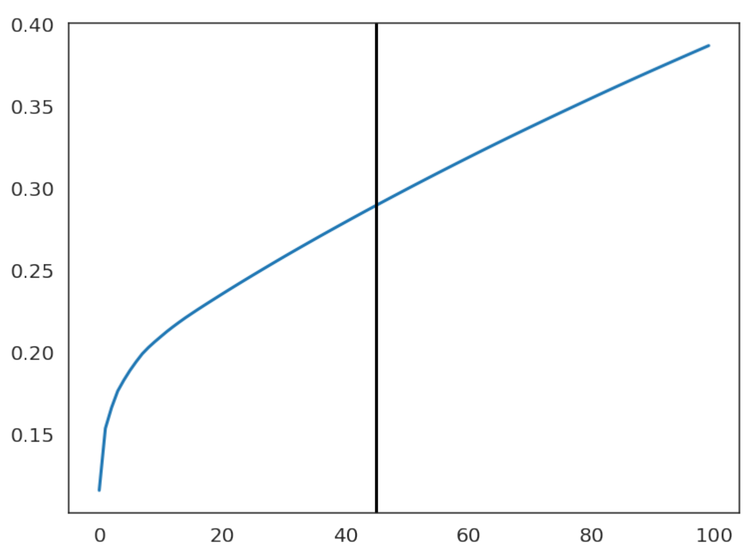
Celloracle uses almost the same strategy as velocyto for visualizing cell transitions. This process requires KNN imputation in advance.

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.
↪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”.

Pleasae 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

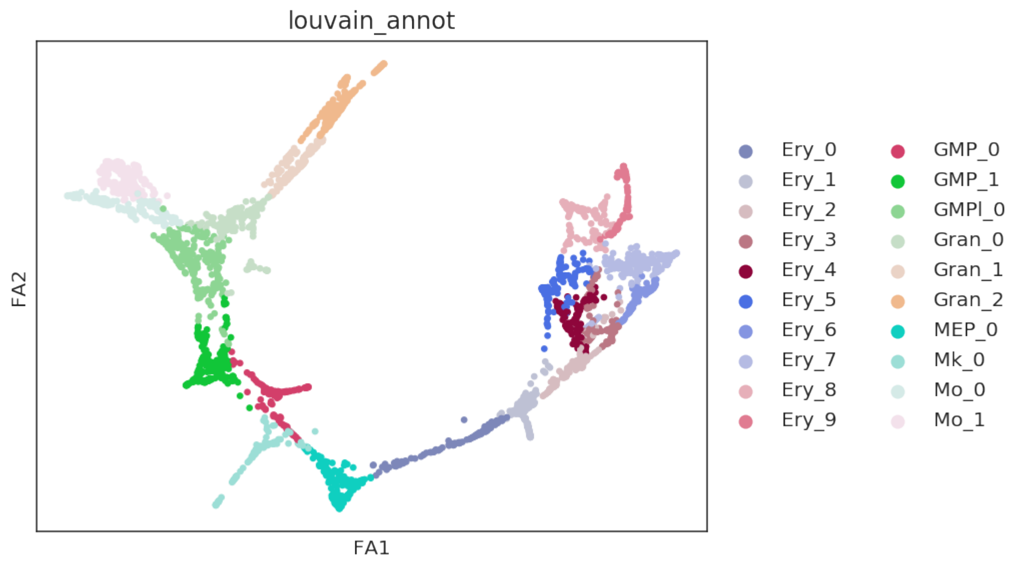
The next step is constructing a cluster-specific GRN for all clusters.

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', 'GMPl_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)

(continued from previous page)

```
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     Nfkb1              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 pallete
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
GMPl_0  #8DD593
Mo_0    #D5EAE7
Mo_1    #F3E1EB
Gran_0  #C6DEC7
Gran_1  #EAD3C6
Gran_2  #F0B98D
```

[76]:
```
# Change the order of pallete
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',
         'GMPl_0', 'Mo_0', 'Mo_1', 'Gran_0', 'Gran_1', 'Gran_2']
links.palette = links.palette.loc[order]
links.palette
```

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

(continues on next page)

(continued from previous page)

```
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
GMPl_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

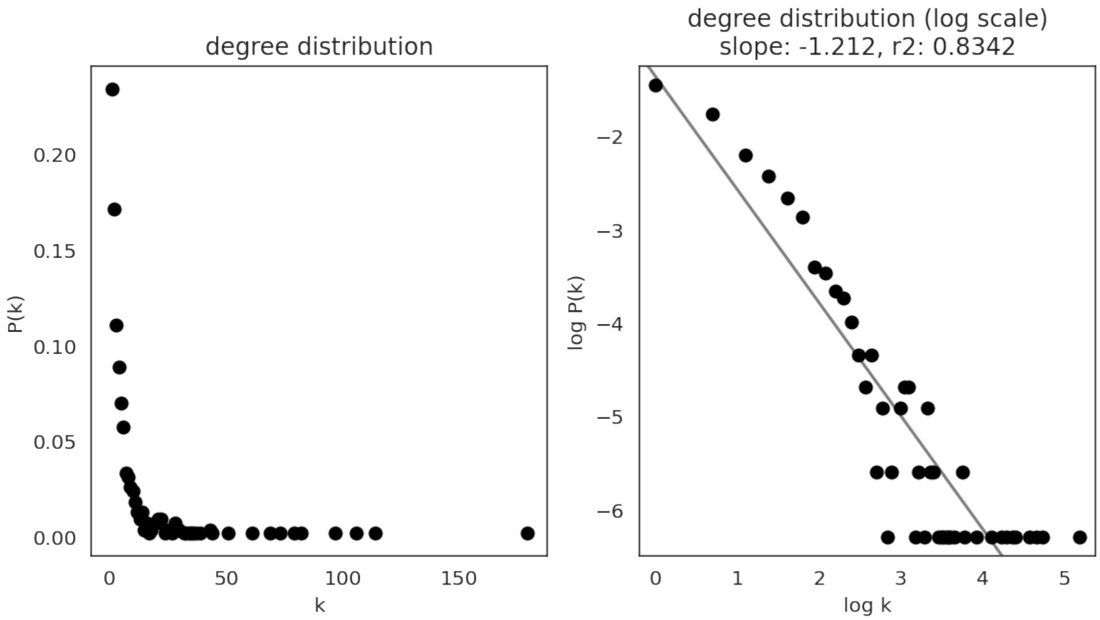
In the first step, we examine the network degree distribution. Network degree, which is the number of edges for each node, is one of the important metrics used to investigate the network structure (https://en.wikipedia.org/wiki/Degree_distribution).

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

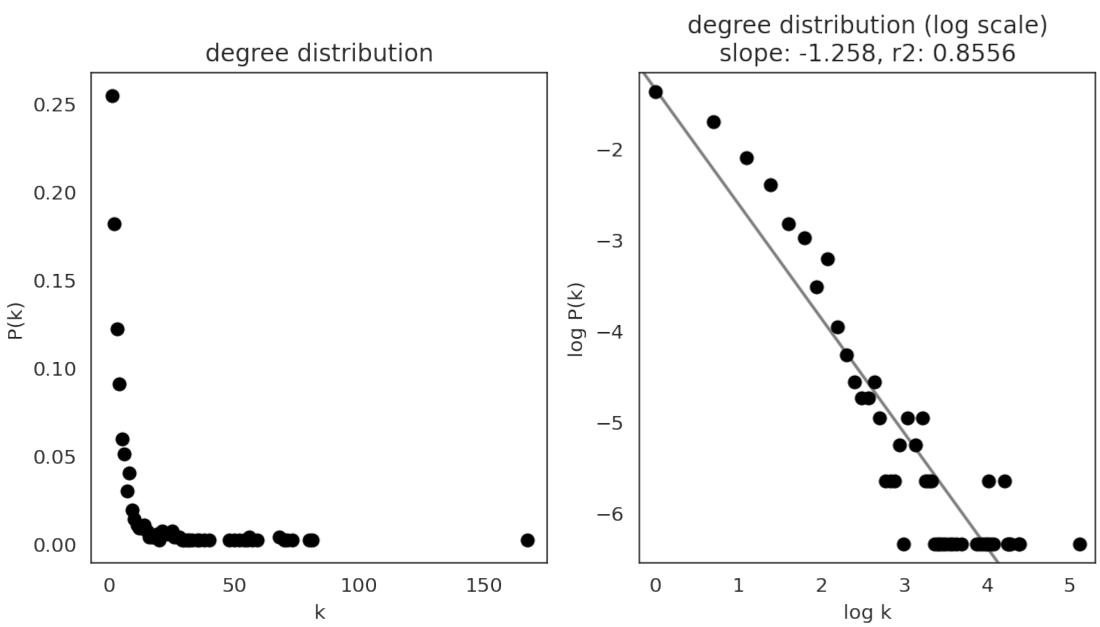
[9]:
```
plt.rcParams["figure.figsize"] = [9, 4.5]
```

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

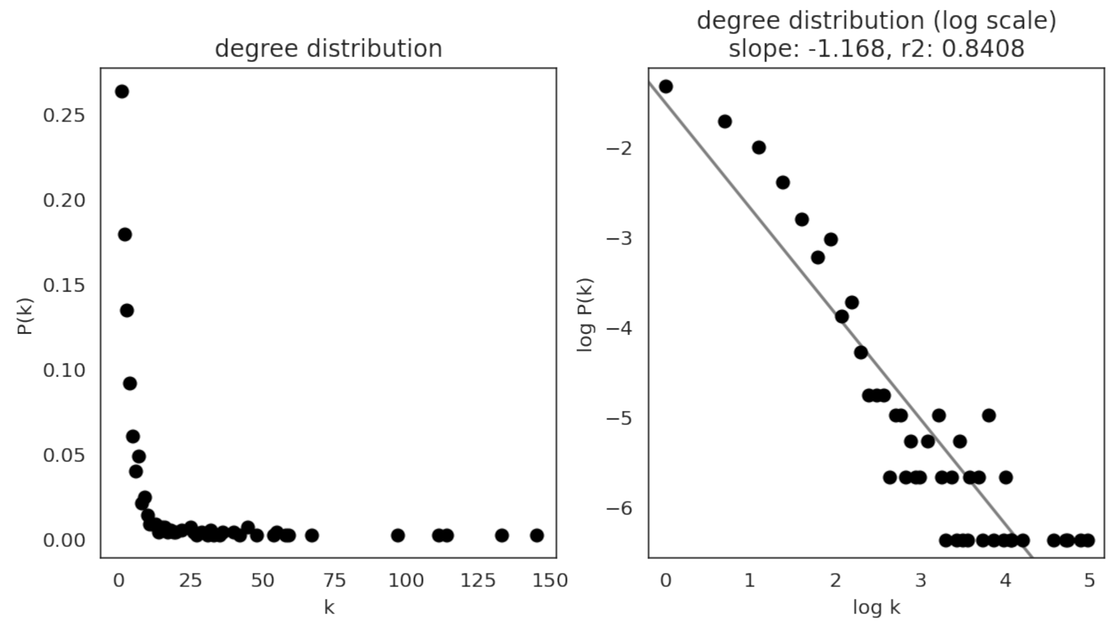
```
Ery_0
```



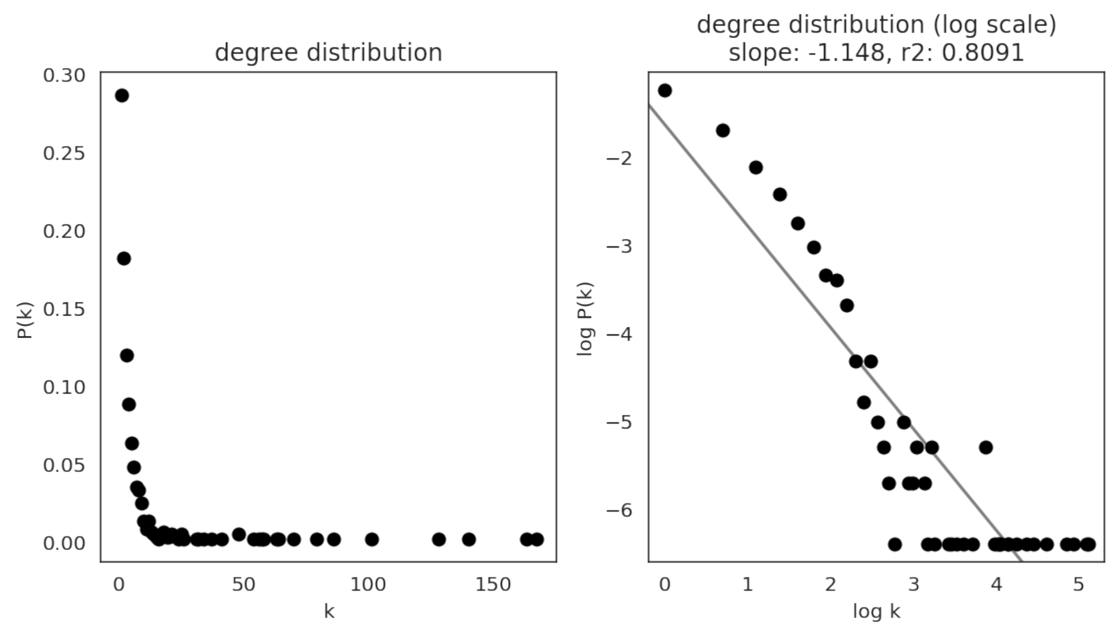

```
Ery_1
```



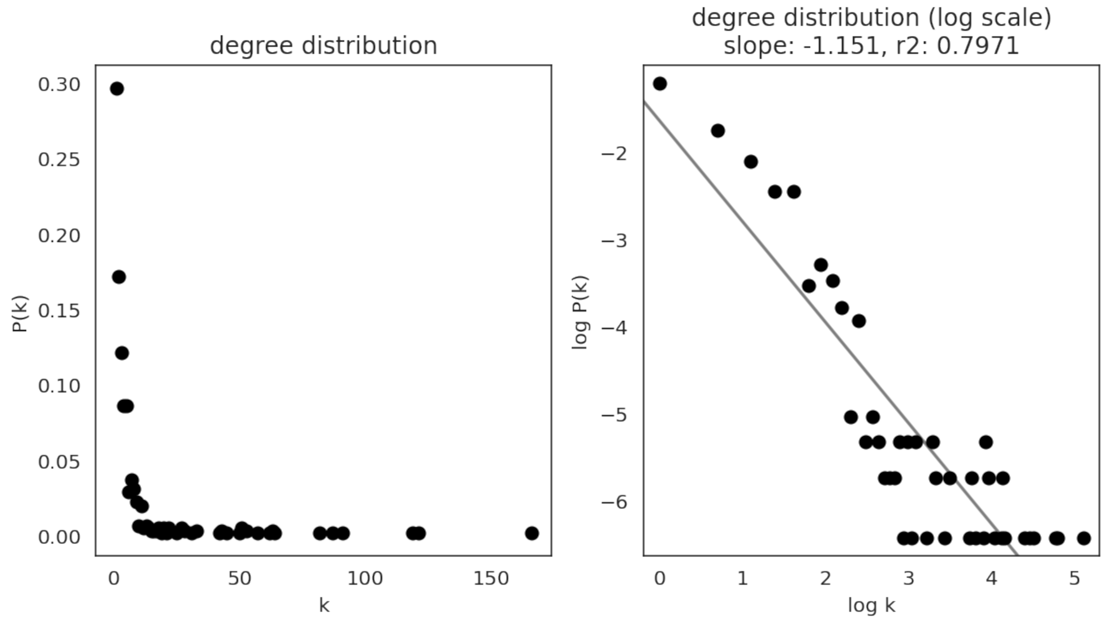

```
Ery_2
```


degree distribution
P(k)
0.25
0.20
0.15
0.10
0.05
0.00
0
25
50
75
100
125
150
k
degree distribution (log scale)
slope: -1.168, r2: 0.8408
log P(k)
−2
−3
−4
−5
−6
0
1
2
3
4
5
log k

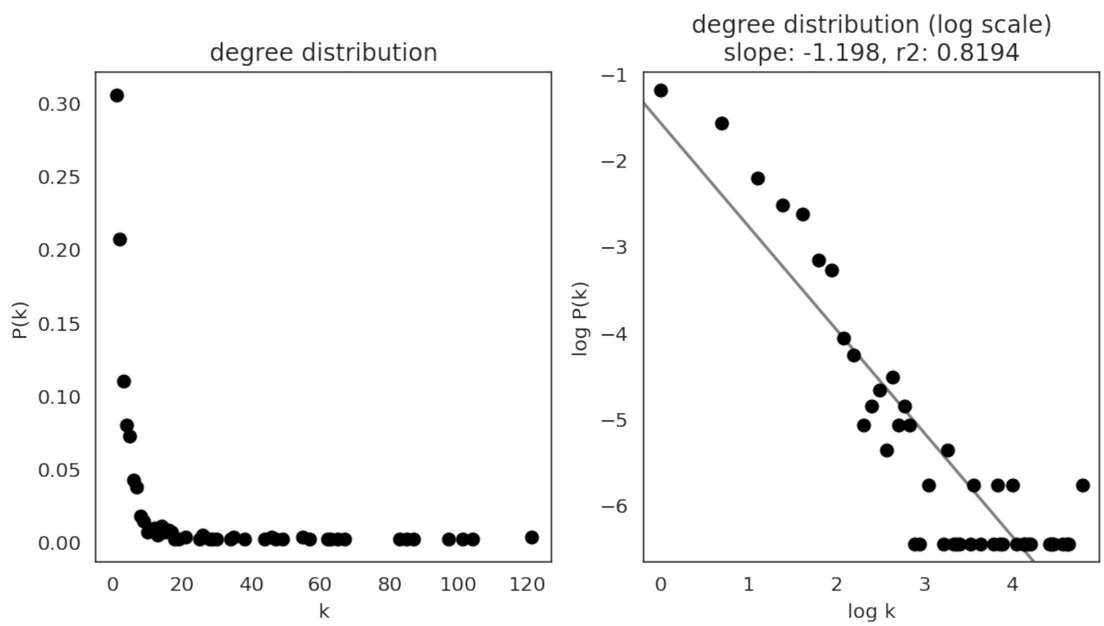
Ery_3


degree distribution
P(k)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
50
100
150
k
degree distribution (log scale)
slope: -1.148, r2: 0.8091
log P(k)
−2
−3
−4
−5
−6
0
1
2
3
4
5
log k

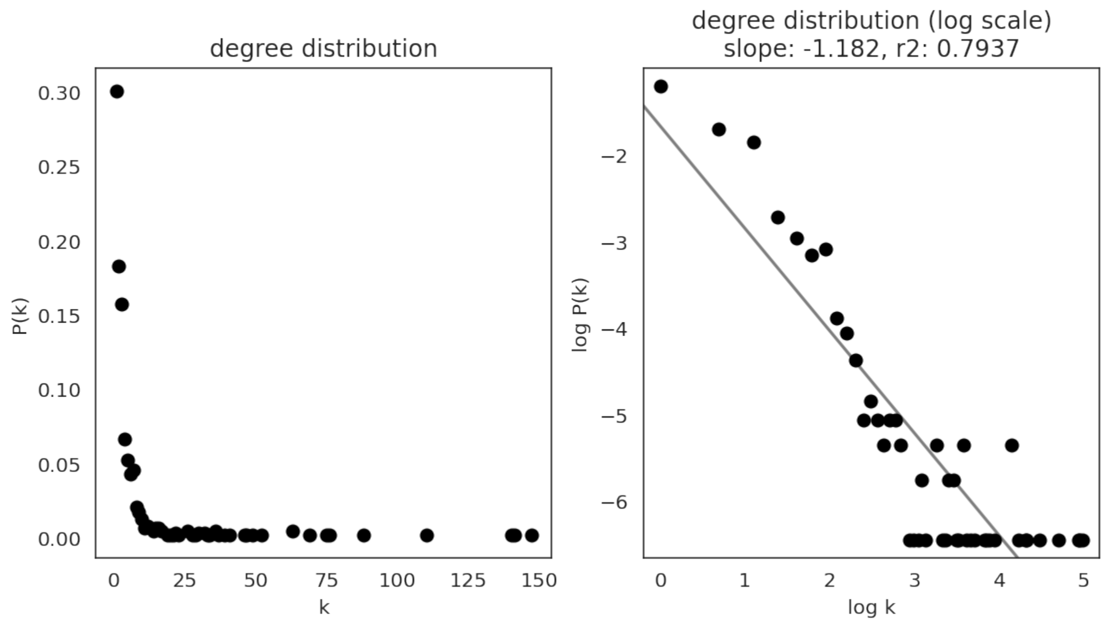
Ery_4


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.151, r2: 0.7971
log P(k)
log k

Ery_5

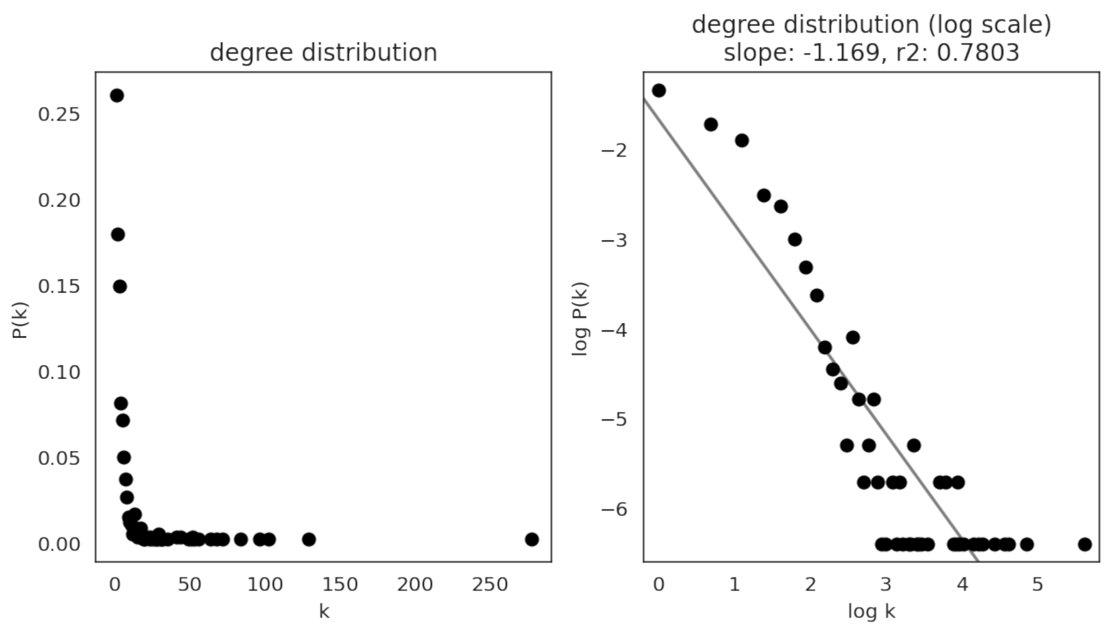

degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.198, r2: 0.8194
log P(k)
log k

Ery_6



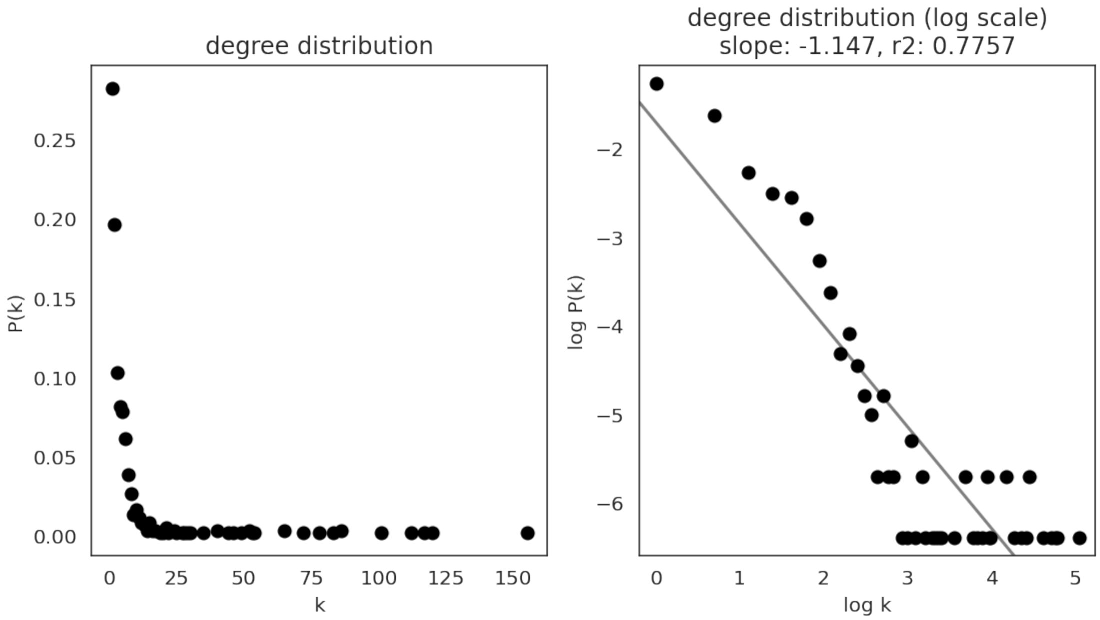
degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.182, r2: 0.7937
log P(k)
log k


Ery_7

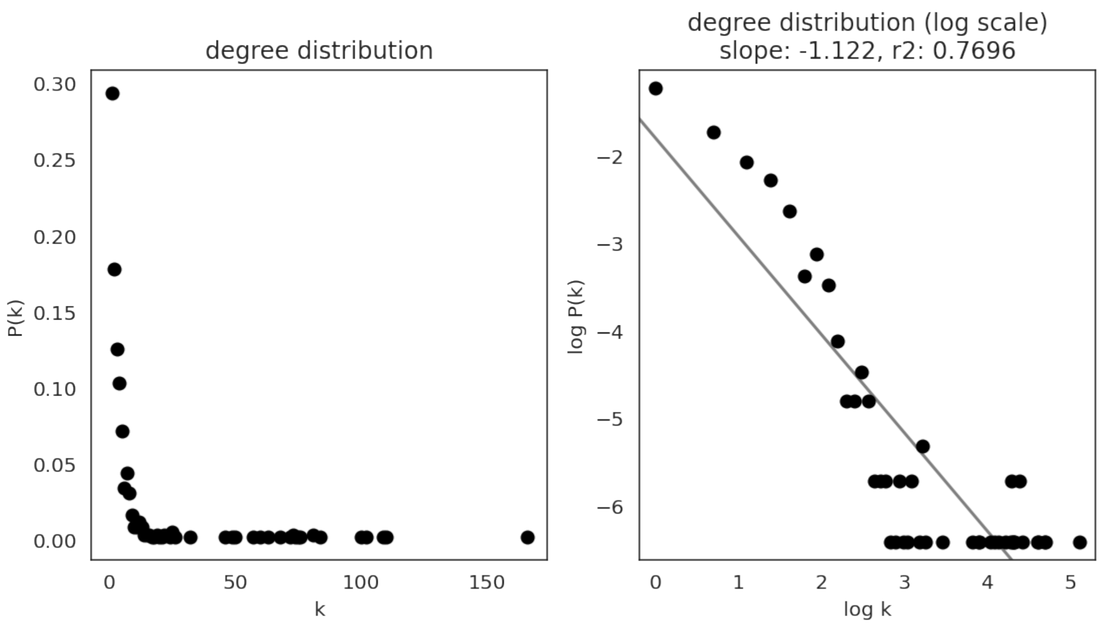


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.169, r2: 0.7803
log P(k)
log k

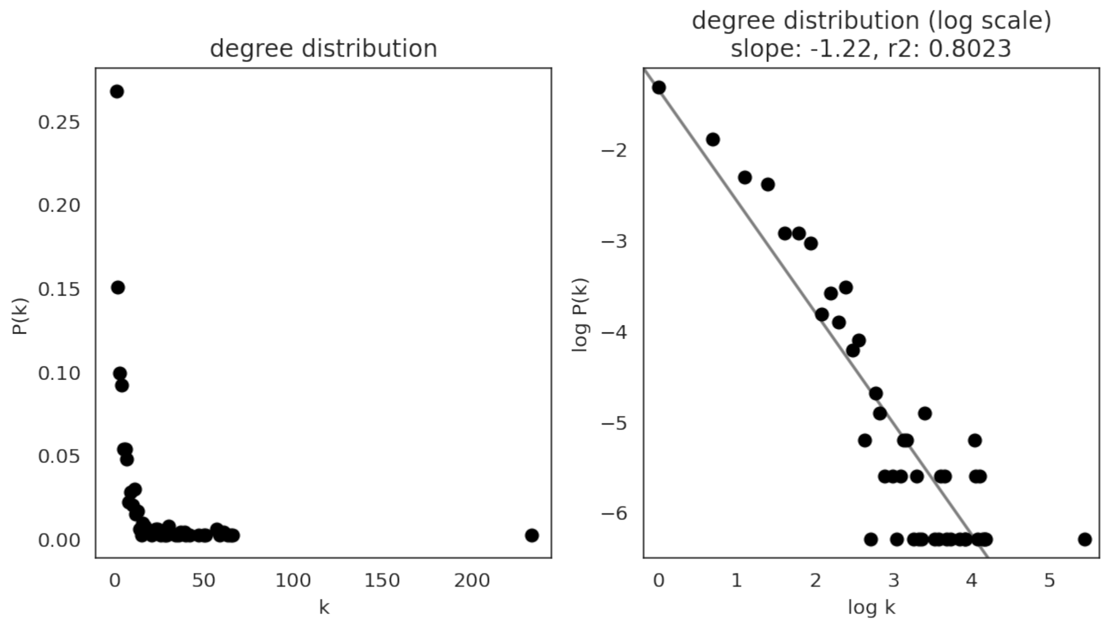

Ery_8


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.147, r2: 0.7757
log P(k)
log k

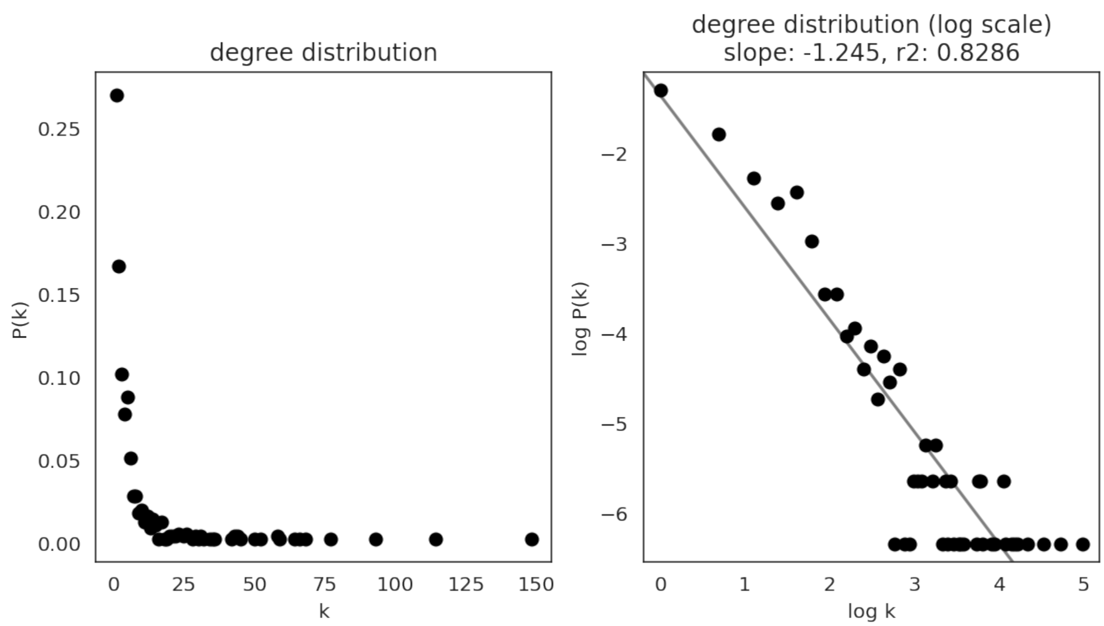
Ery_9


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.122, r2: 0.7696
log P(k)
log k

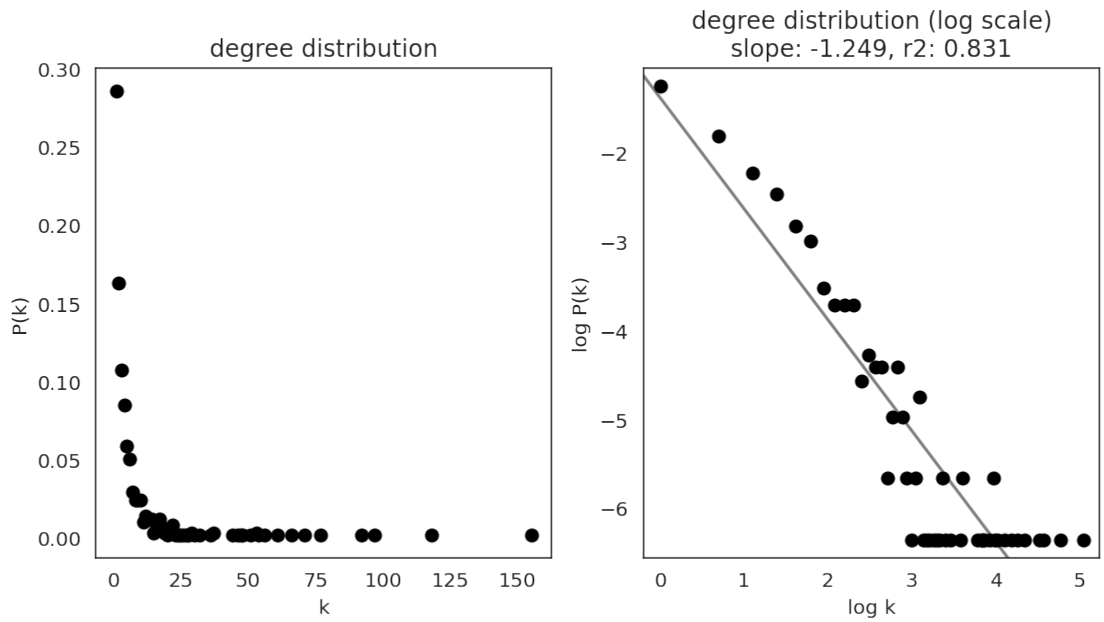
GMP_0


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.22, r2: 0.8023
log P(k)
log k

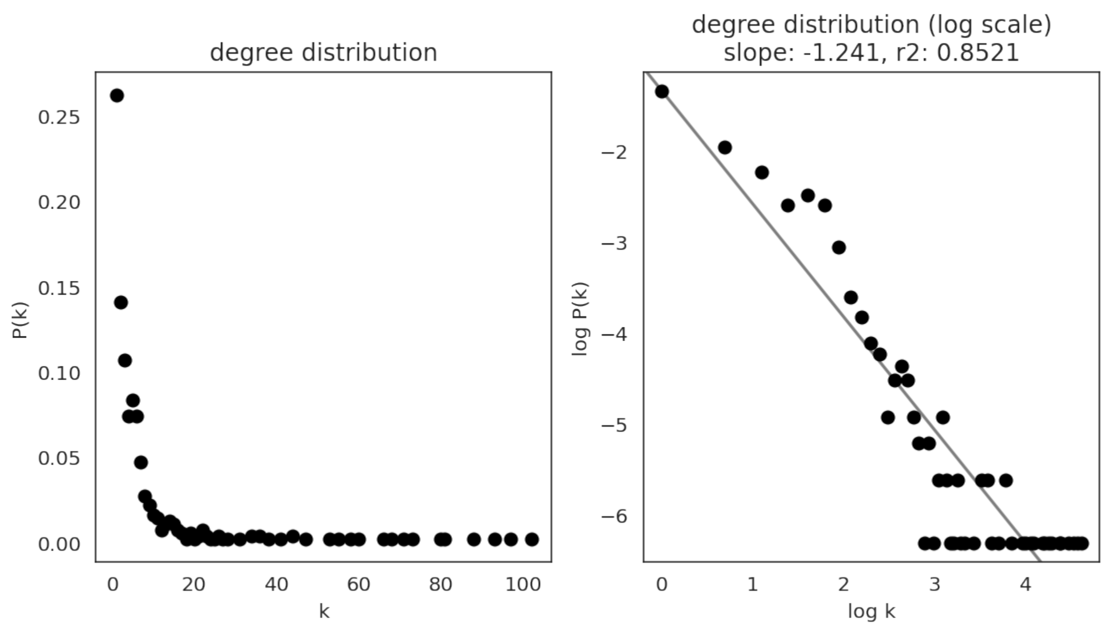
GMP_1


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.245, r2: 0.8286
log P(k)
log k

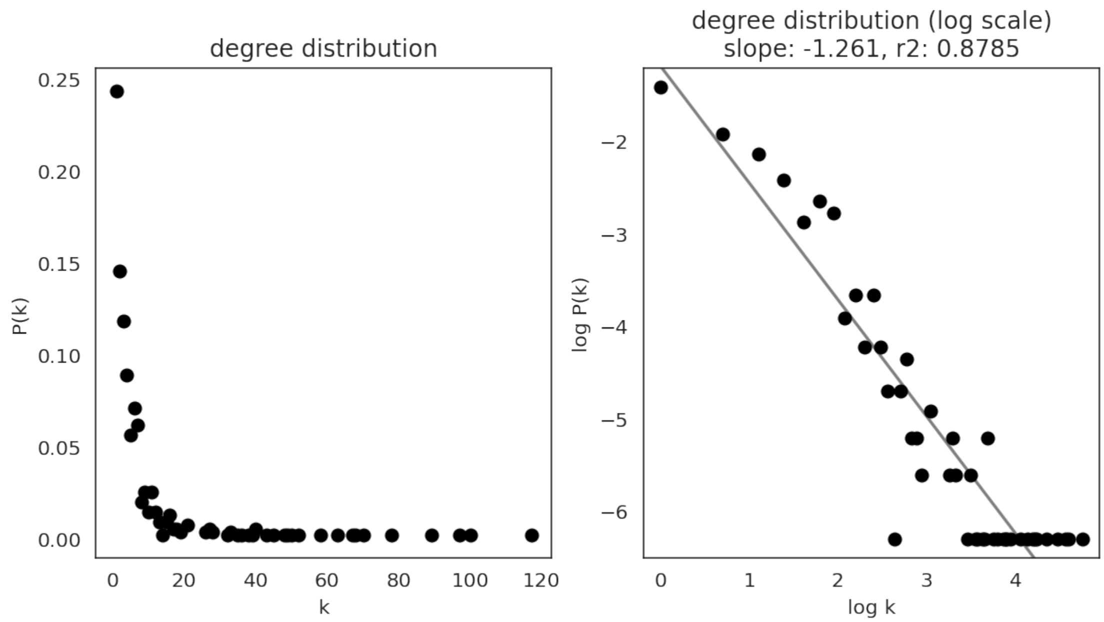
GMP1_0


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.249, r2: 0.831
log P(k)
log k

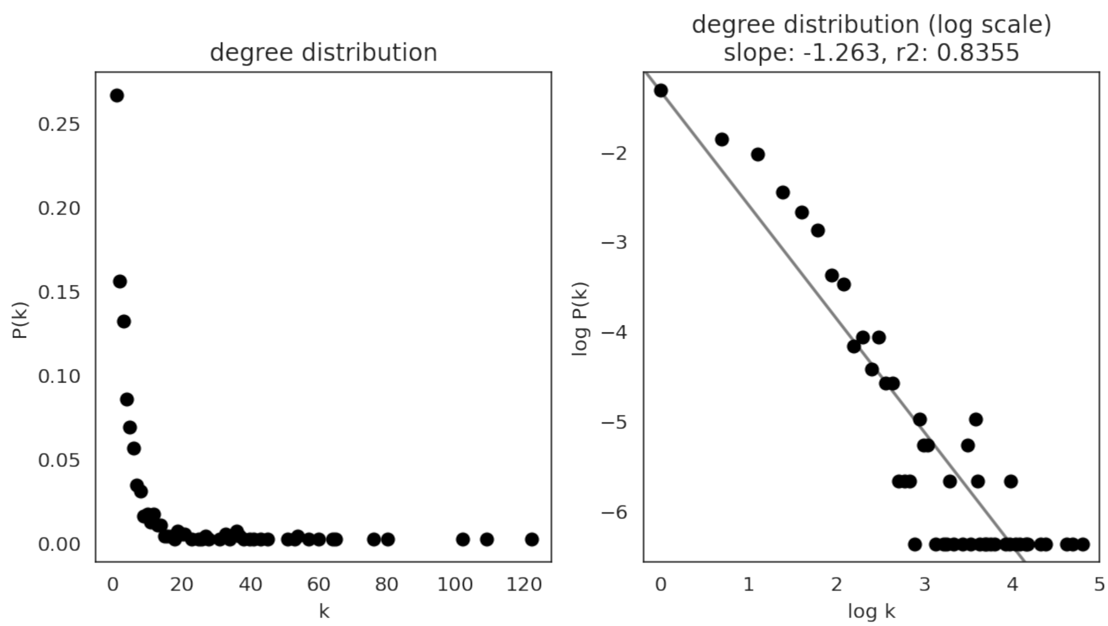
Gran_0


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.241, r2: 0.8521
log P(k)
log k

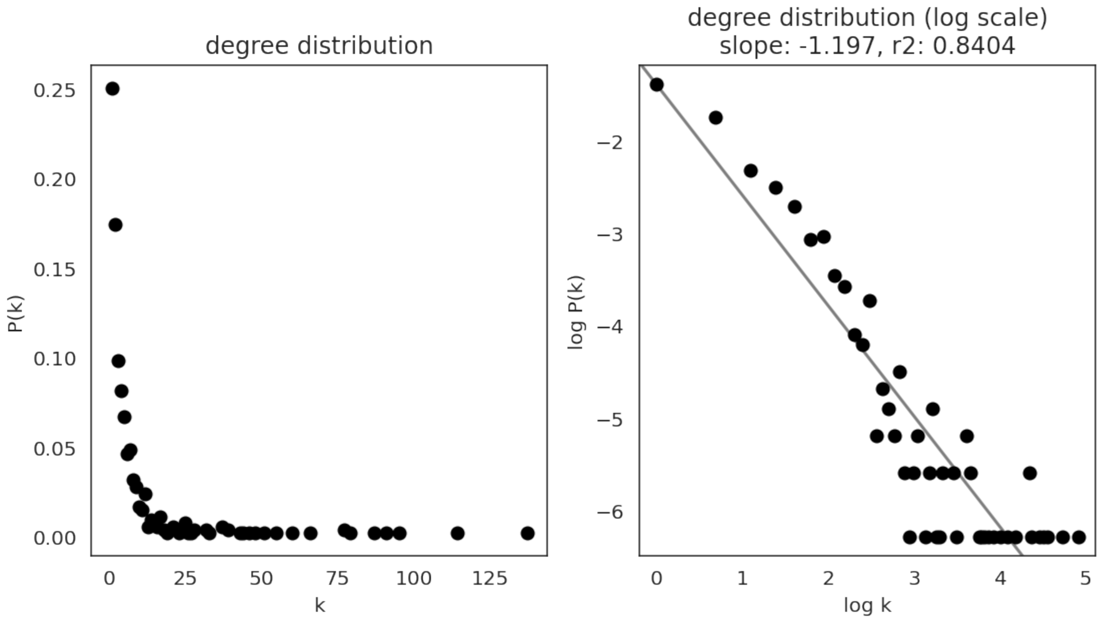
Gran_1


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.261, r2: 0.8785
log P(k)
log k

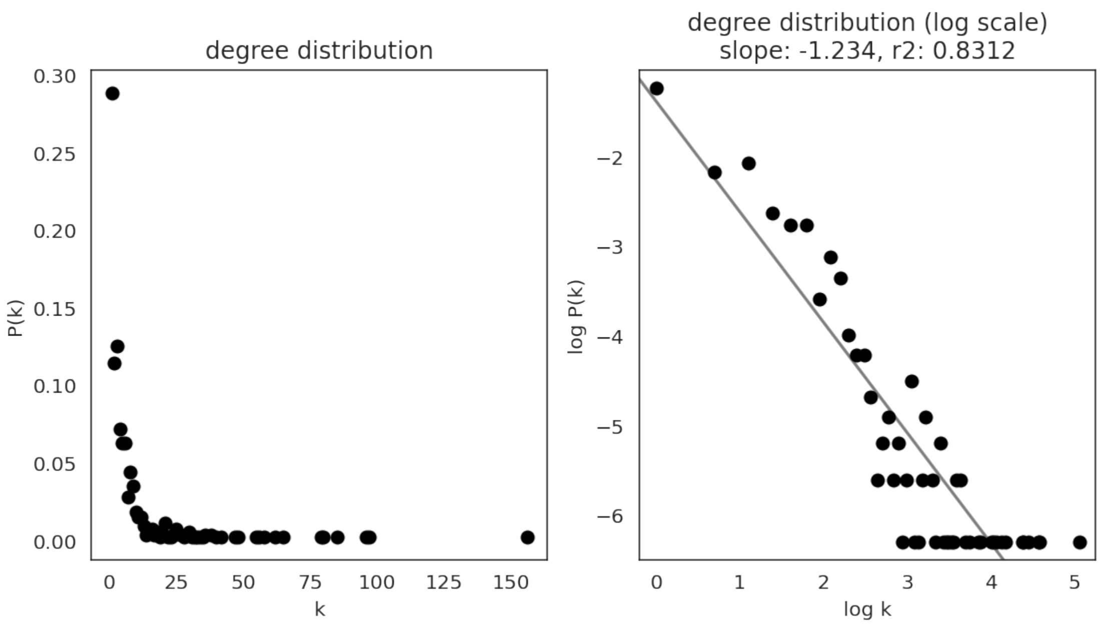
Gran_2


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.263, r2: 0.8355
log P(k)
log k

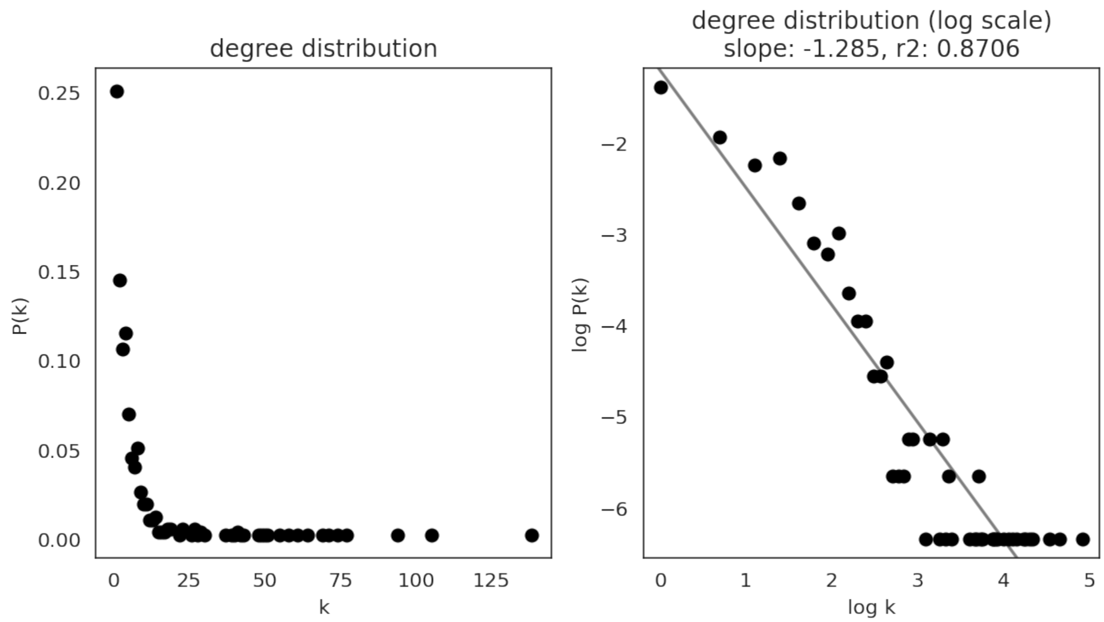
MEP_0


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.197, r2: 0.8404
log P(k)
log k

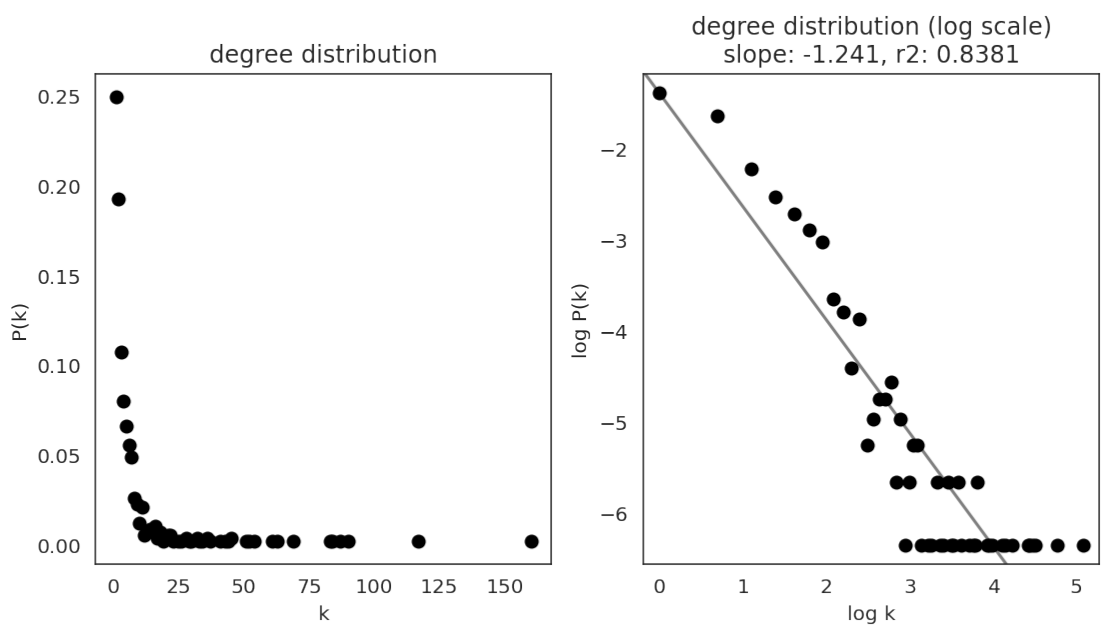
Mk_0


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.234, r2: 0.8312
log P(k)
log k

Mo_0




Mo_1




[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.
GMPl_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  \
```

(continues on next page)

(continued from previous page)

```
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       0.647531  Connector Hub   Ery_0
Mycn        2      1.767680       0.595556     Peripheral   Ery_0
E2f4        0      8.575037       0.632252  Connector Hub   Ery_0
Zbtb1       2      1.317952       0.533058     Peripheral   Ery_0
Ybx1        3      5.306800       0.687251  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
```

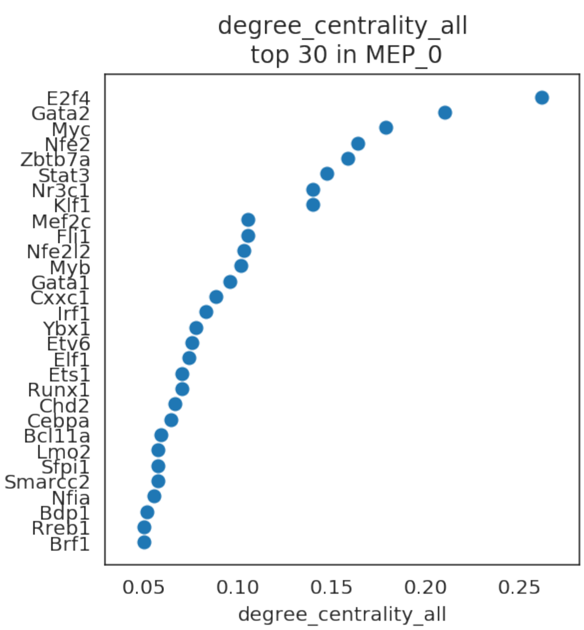
[83]:
```
['Ery_0',
 'Ery_1',
 'Ery_2',
 'Ery_3',
 'Ery_4',
 'Ery_5',
```

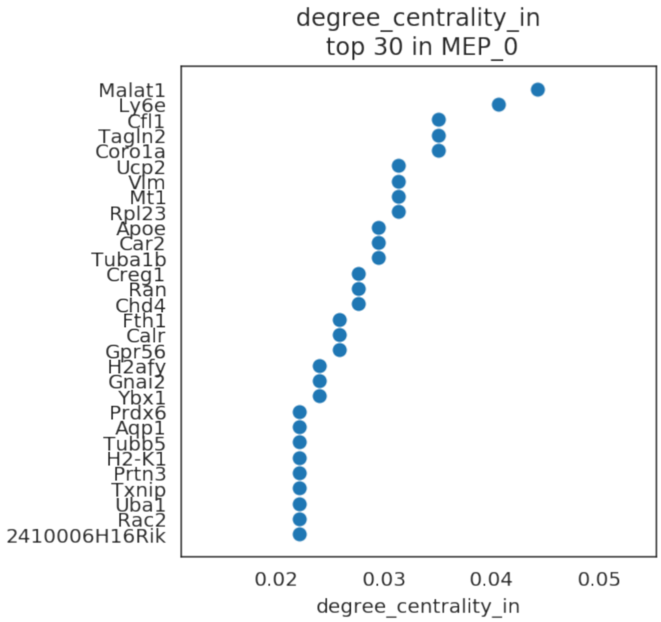
(continues on next page)

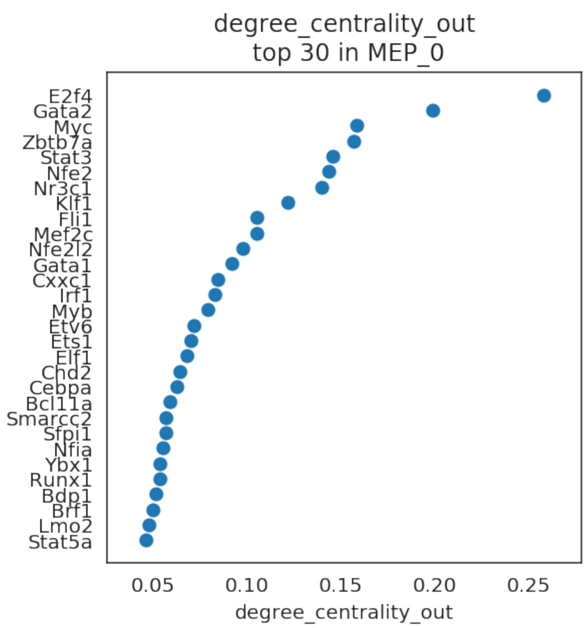
(continued from previous page)

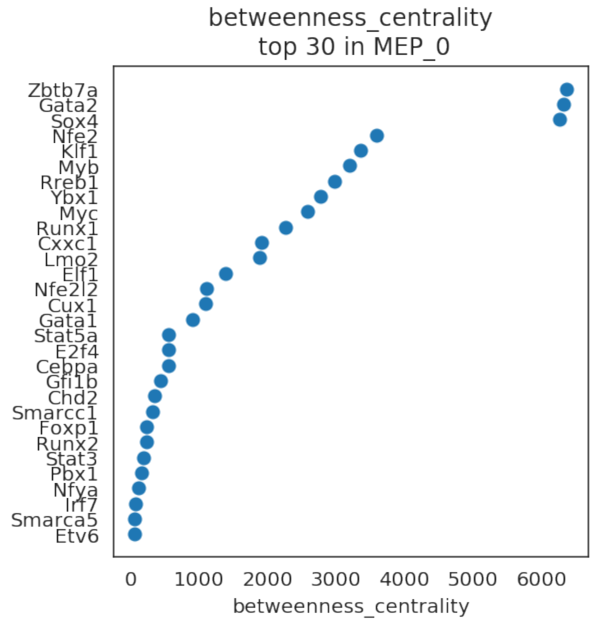
```
 'Ery_6',
 'Ery_7',
 'Ery_8',
 'Ery_9',
 'GMP_0',
 'GMP_1',
 'GMPl_0',
 'Gran_0',
 'Gran_1',
 'Gran_2',
 'MEP_0',
 'Mk_0',
 'Mo_0',
 'Mo_1']
```

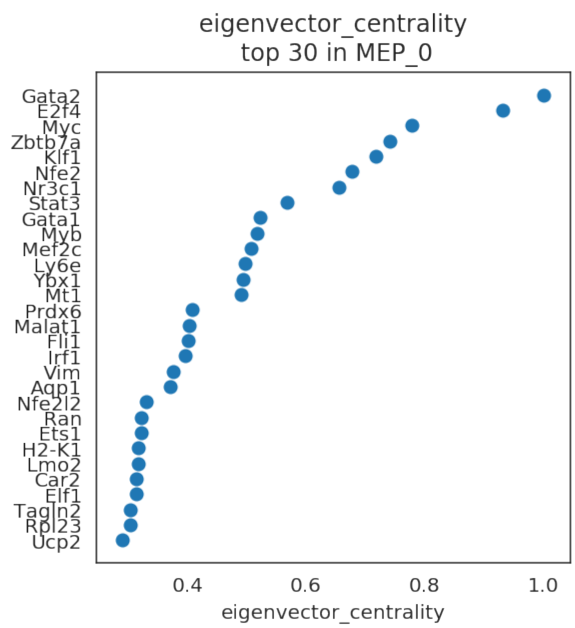
[53]:
```
# Visualize top n-th genes that have high scores.
links.plot_scores_as_rank(cluster="MEP_0", n_gene=30, save=f"{save_folder}/ranked_
↪score")
```

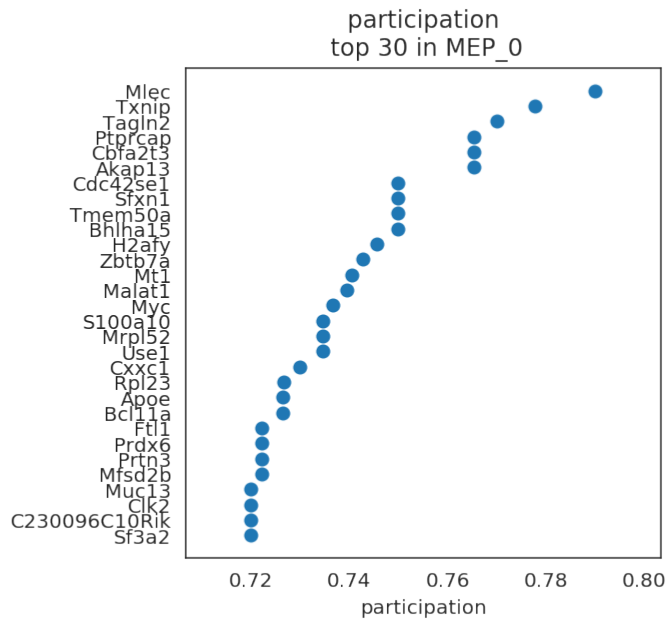



degree_centrality_in
top 30 in MEP_0
Malat1
Ly6e
Cfl1
Tagln2
Coro1a
Ucp2
Vim
Mt1
Rpl23
Apoe
Car2
Tuba1b
Creg1
Ran
Chd4
Fth1
Calr
Gpr56
H2afy
Gnai2
Ybx1
Prdx6
Aqp1
Tubb5
H2-K1
Prtn3
Txnip
Uba1
Rac2
2410006H16Rik
0.02
0.03
0.04
0.05
degree_centrality_in


degree_centrality_out
top 30 in MEP_0
E2f4
Gata2
Myc
Zbtb7a
Stat3
Nfe2
Nr3c1
Klf1
Fli1
Mef2c
Nfe2l2
Gata1
Cxxc1
Irf1
Myb
Etv6
Ets1
Elf1
Chd2
Cebpa
Bcl11a
Smarcc2
Sfpi1
Nfia
Ybx1
Runx1
Bdp1
Brf1
Lmo2
Stat5a
0.05
0.10
0.15
0.20
0.25
degree_centrality_out


betweenness_centrality
top 30 in MEP_0
Zbtb7a
Gata2
Sox4
Nfe2
Klf1
Myb
Rreb1
Ybx1
Myc
Runx1
Cxxc1
Lmo2
Elf1
Nfe2l2
Cux1
Gata1
Stat5a
E2f4
Cebpa
Gfi1b
Chd2
Smarcc1
Foxp1
Runx2
Stat3
Pbx1
Nfya
Irf7
Smarca5
Etv6
0
1000
2000
3000
4000
5000
6000
betweenness_centrality


eigenvector_centrality
top 30 in MEP_0
Gata2
E2f4
Myc
Zbtb7a
Klf1
Nfe2
Nr3c1
Stat3
Gata1
Myb
Mef2c
Ly6e
Ybx1
Mt1
Prdx6
Malat1
Fli1
Irf1
Vim
Aqp1
Nfe2l2
Ran
Ets1
H2-K1
Lmo2
Car2
Elf1
Tagln2
Rpl23
Ucp2
0.4
0.6
0.8
1.0
eigenvector_centrality

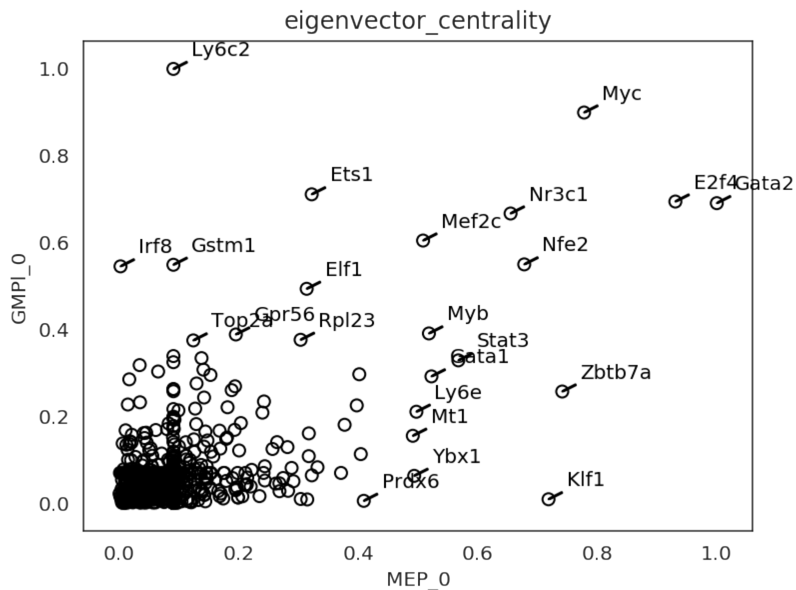



## 7.2. Network score comparison between two clusters

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")
```



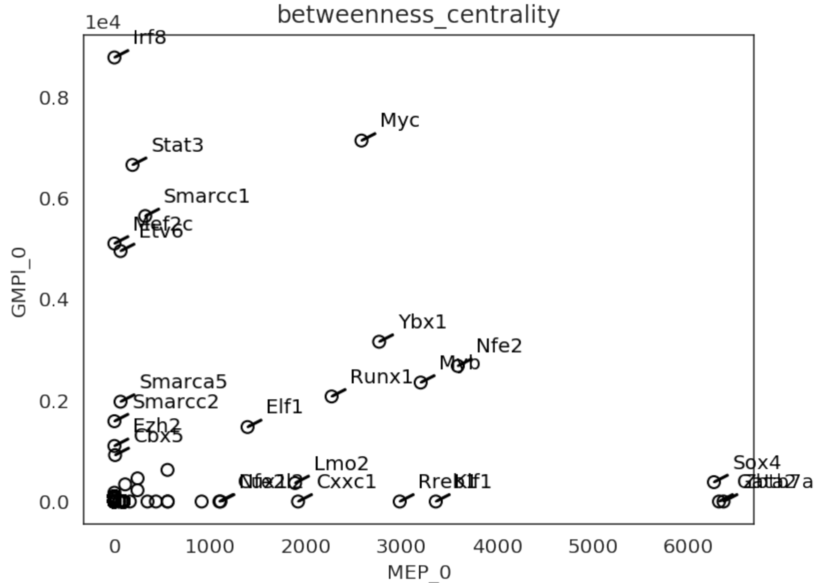

[55]:

```
plt.ticklabel_format(style='sci',axis='y',scilimits=(0,0))
```

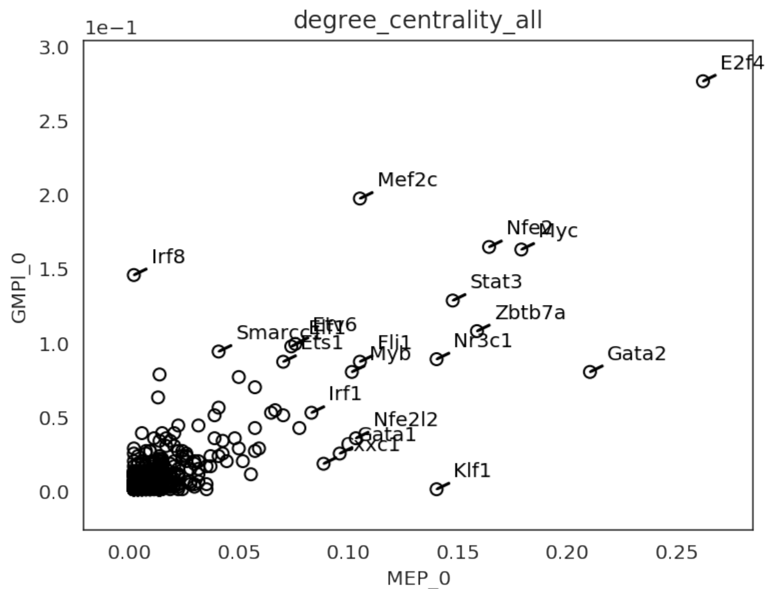
(continues on next page)

(continued from previous page)

```
links.plot_score_comparison_2D(value="betweenness_centrality",
                               cluster1="MEP_0", cluster2="GMPl_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="GMPl_0",
                               percentile=98, save=f"{save_folder}/score_comparison")
```



### 7.3. Network score dynamics

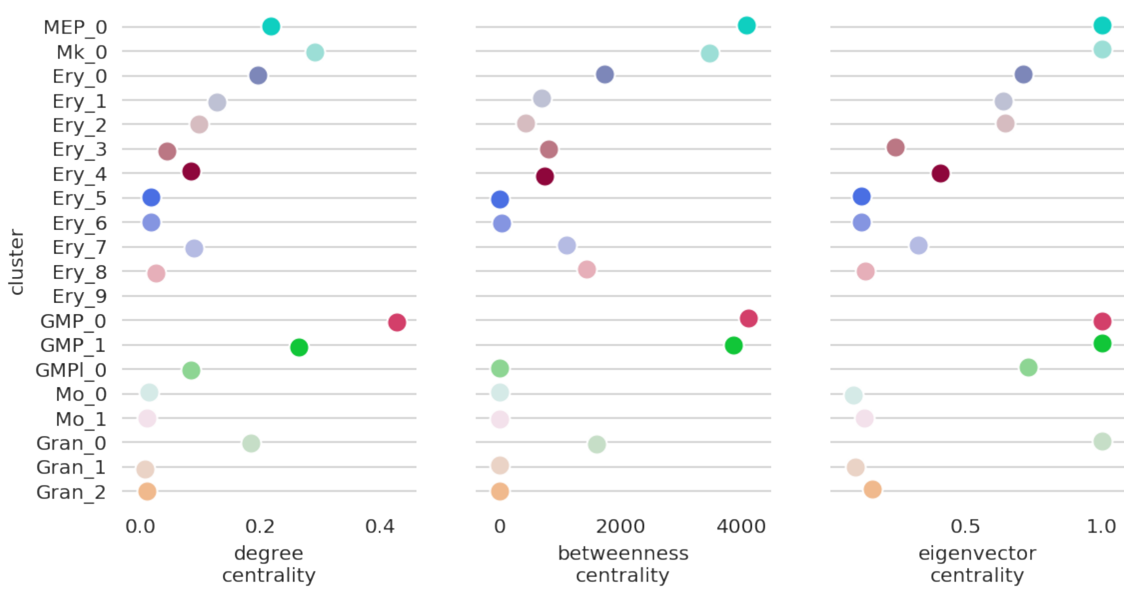
In the following session, we focus on how a gene's network score changes during the differentiation.

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. .

[30]:
```
# Visualize Gata2 network score dynamics
links.plot_score_per_cluster(goi="Gata2", save=f"{save_folder}/network_score_per_gene/
↪")
```

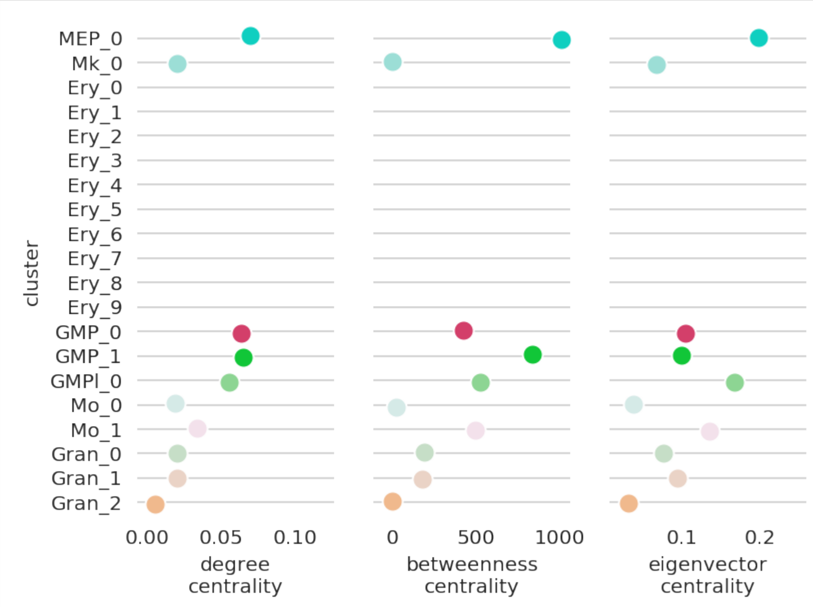
```
Gata2
```




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 erythloids clusters, and there is no degree scores for Cebpa in these clusters as follows.

[38]:
```
links.plot_score_per_cluster(goi="Cebpa")
```

```
Cebpa
```




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()
```

```
[39]:       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"]
```

```
[41]: 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.

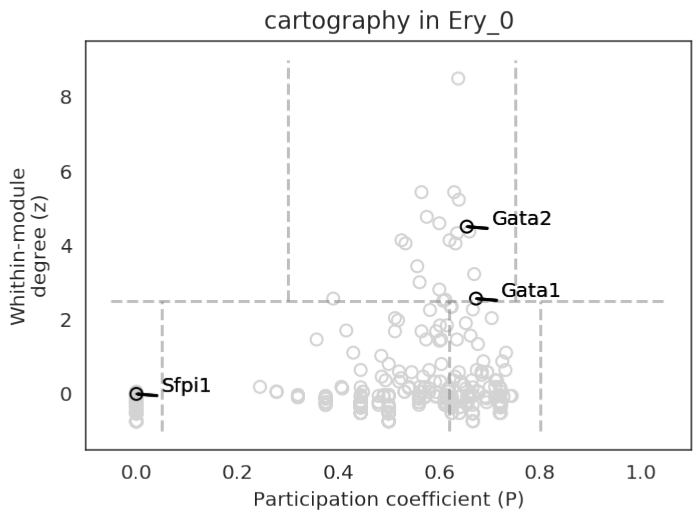
```
[58]: # Plot cartography as a scatter plot
      links.plot_cartography_scatter_per_cluster(scatter=True,
                                                 kde=False,
                                                 gois=["Gata1", "Gata2", "Sfpi1"],
```

(continues on next page)

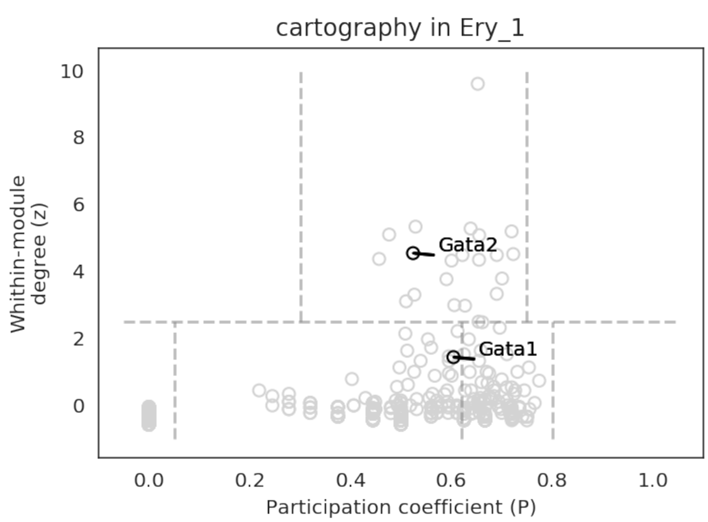
(continued from previous page)

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

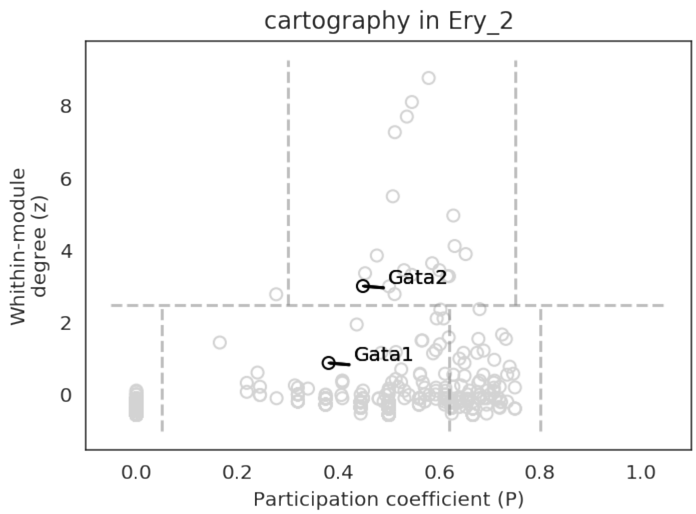
```
Ery_0
```



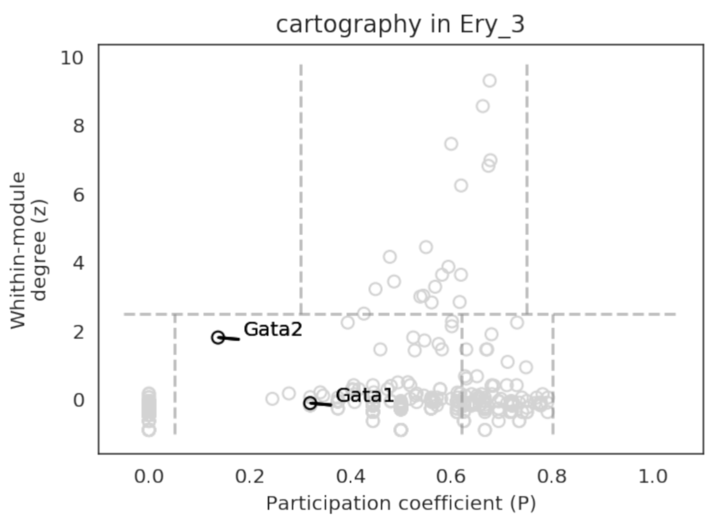

```
Ery_1
```



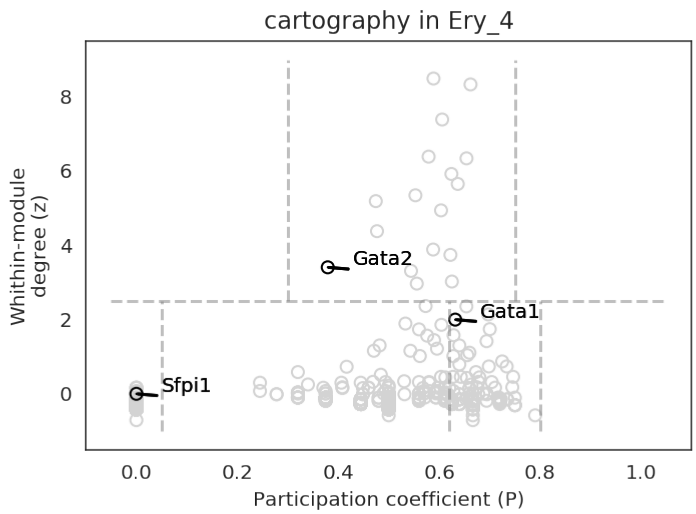

```
Ery_2
```


cartography in Ery_2
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Gata1
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8

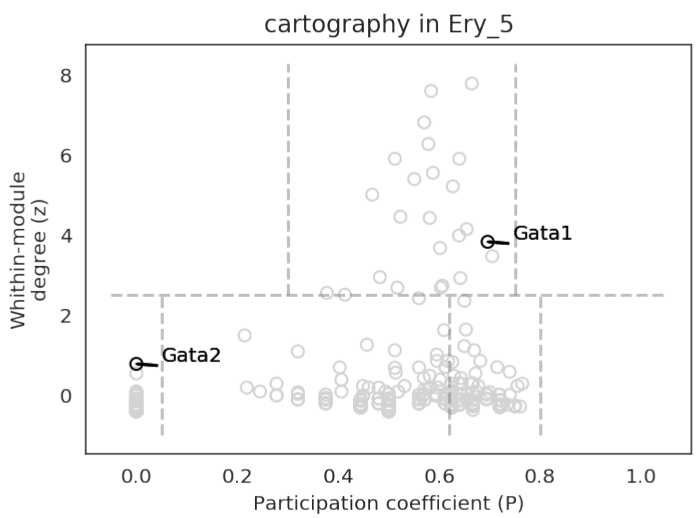
```
Ery_3
```


cartography in Ery_3
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Gata1
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10

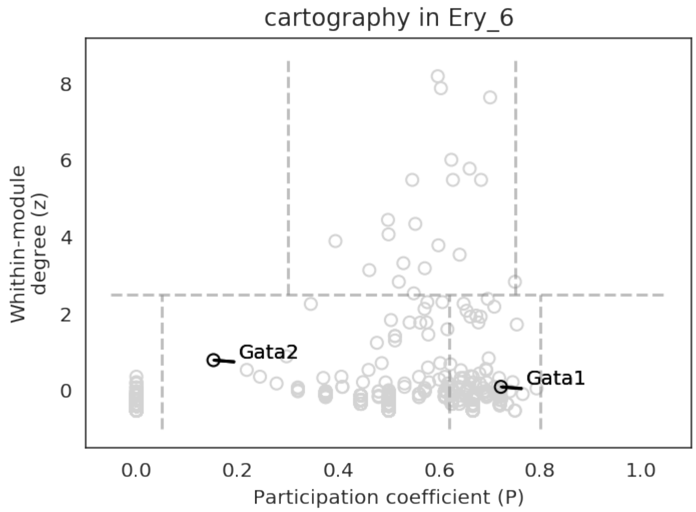
```
Ery_4
```


cartography in Ery_4
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Gata1
Sfpi1
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0

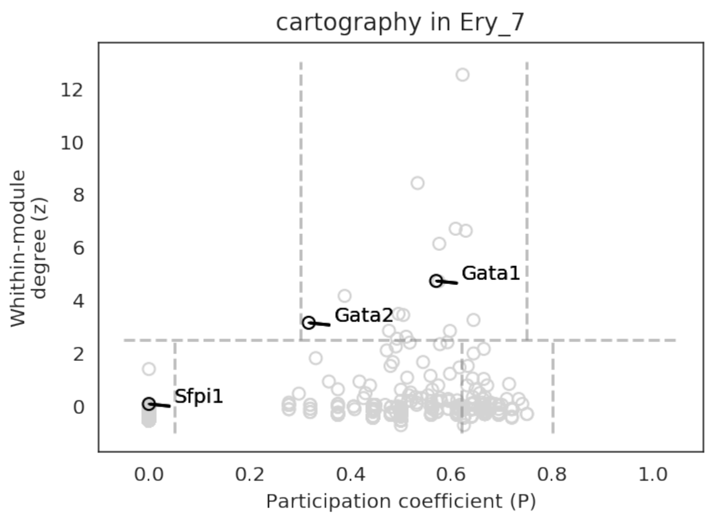
```
Ery_5
```


cartography in Ery_5
Whithin-module degree (z)
Participation coefficient (P)
Gata1
Gata2
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0

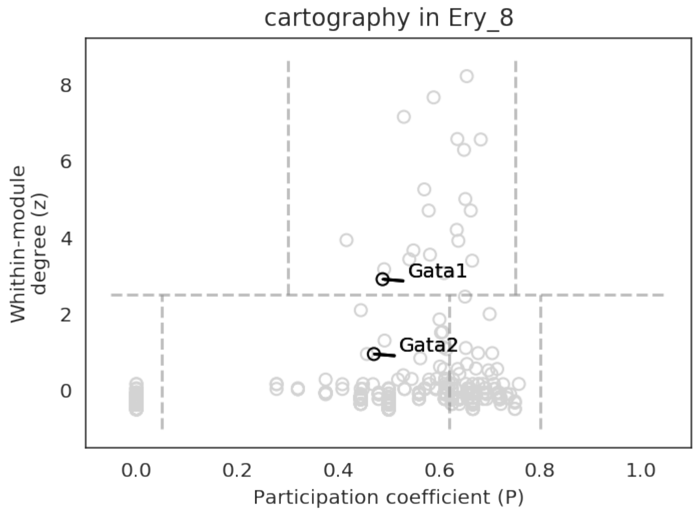
```
Ery_6
```


cartography in Ery_6
Whithin-module
degree (z)
8
6
4
2
0
Gata2
Gata1
0.0
0.2
0.4
0.6
0.8
1.0
Participation coefficient (P)

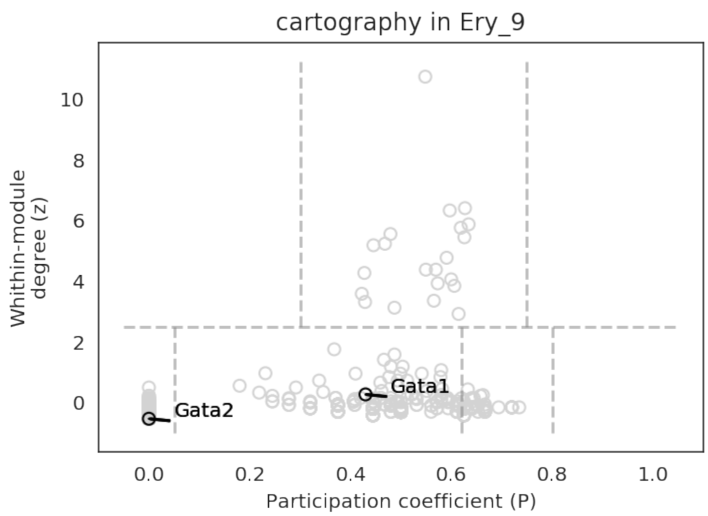
```
Ery_7
```


cartography in Ery_7
Whithin-module
degree (z)
12
10
8
6
4
2
0
Gata1
Gata2
Sfpi1
0.0
0.2
0.4
0.6
0.8
1.0
Participation coefficient (P)

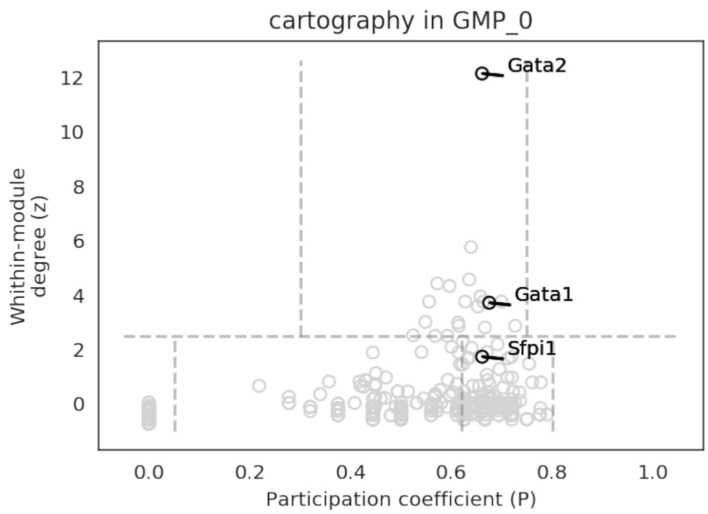
```
Ery_8
```


cartography in Ery_8
Whithin-module degree (z)
Participation coefficient (P)
Gata1
Gata2
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0

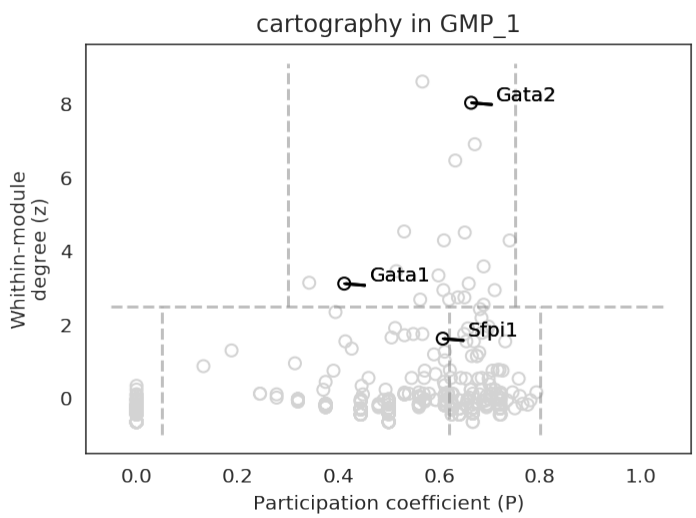
Ery_9


cartography in Ery_9
Whithin-module degree (z)
Participation coefficient (P)
Gata1
Gata2
0
2
4
6
8
10
0.0
0.2
0.4
0.6
0.8
1.0

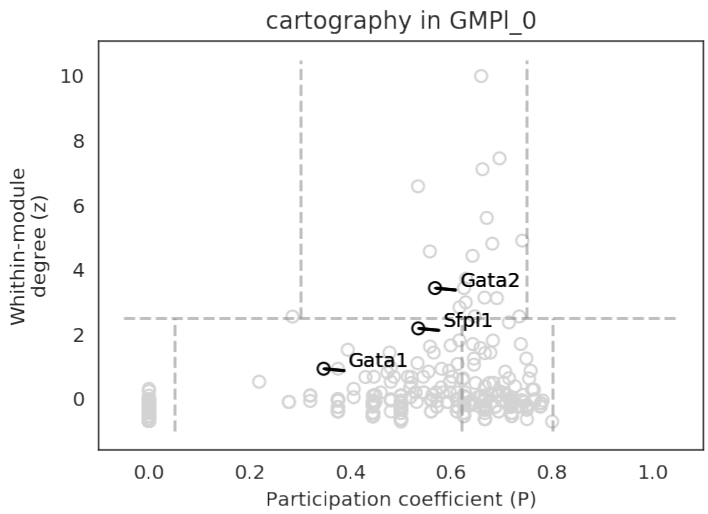
GMP_0


cartography in GMP_0
Gata2
Gata1
Sfpi1
Whithin-module degree (z)
Participation coefficient (P)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
12

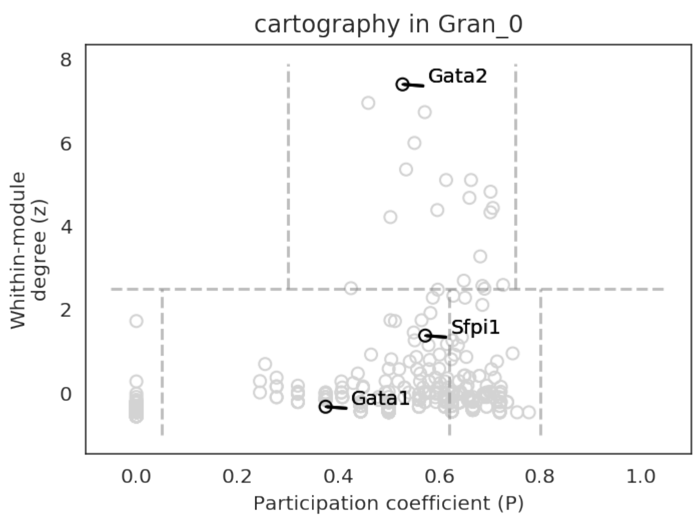
GMP_1


cartography in GMP_1
Gata2
Gata1
Sfpi1
Whithin-module degree (z)
Participation coefficient (P)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8

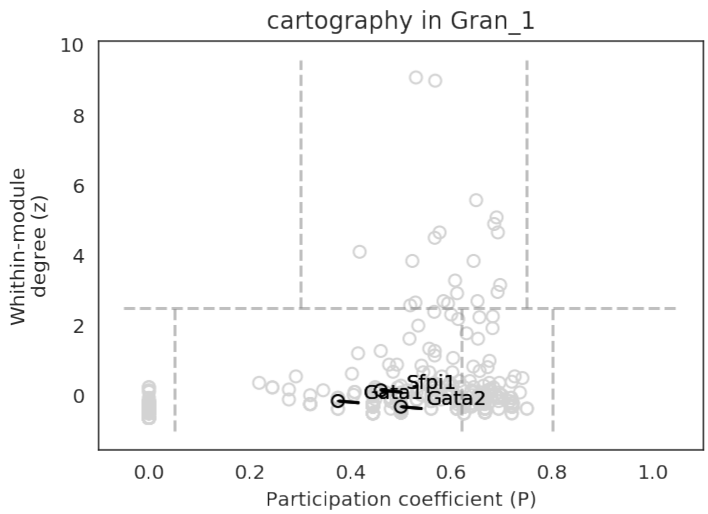
GMP1_0


cartography in GMPl_0
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Sfpi1
Gata1

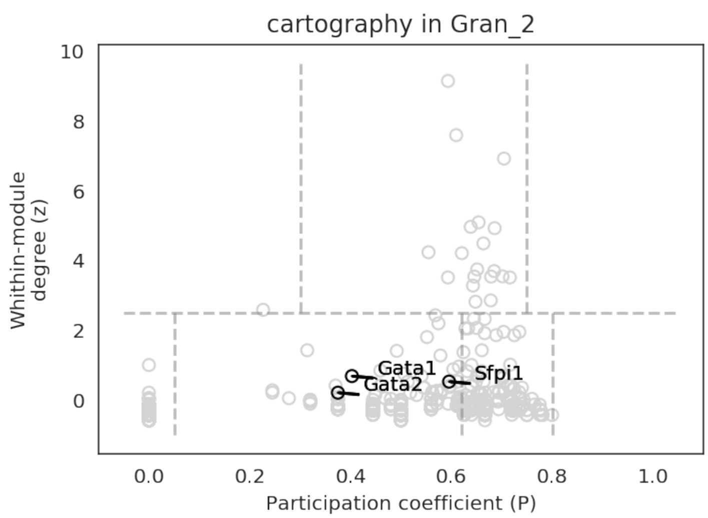
Gran_0


cartography in Gran_0
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Sfpi1
Gata1

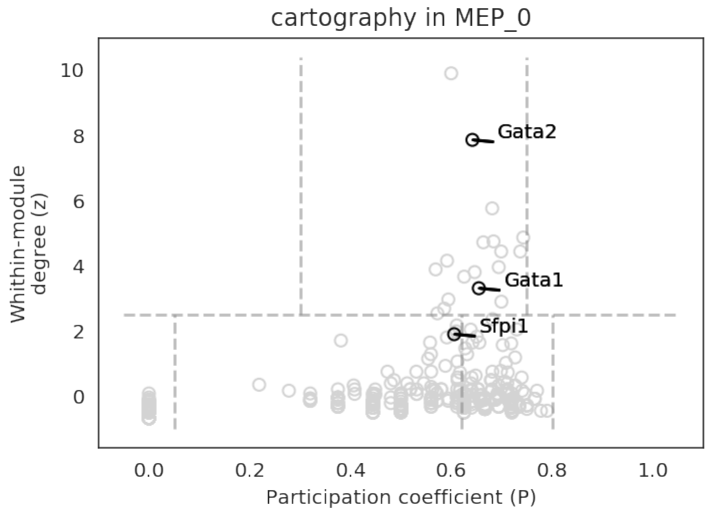
Gran_1


cartography in Gran_1
Whithin-module degree (z)
Participation coefficient (P)
Sfpi1
Gata1
Gata2
10
8
6
4
2
0
0.0
0.2
0.4
0.6
0.8
1.0

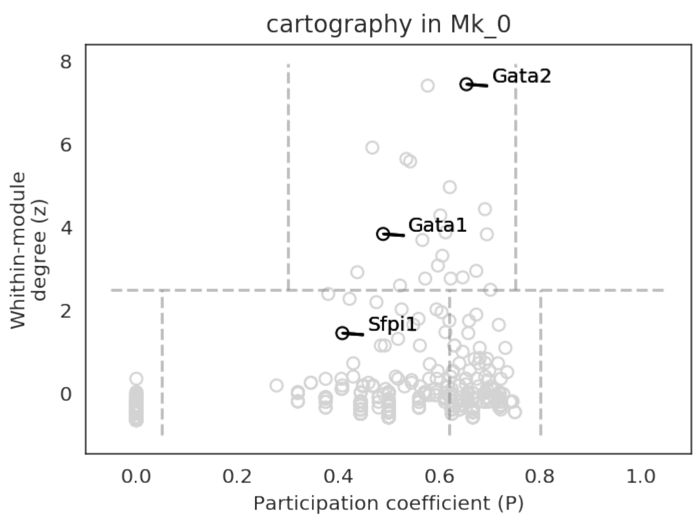
```
Gran_2
```


cartography in Gran_2
Whithin-module degree (z)
Participation coefficient (P)
Gata1
Gata2
Sfpi1
10
8
6
4
2
0
0.0
0.2
0.4
0.6
0.8
1.0

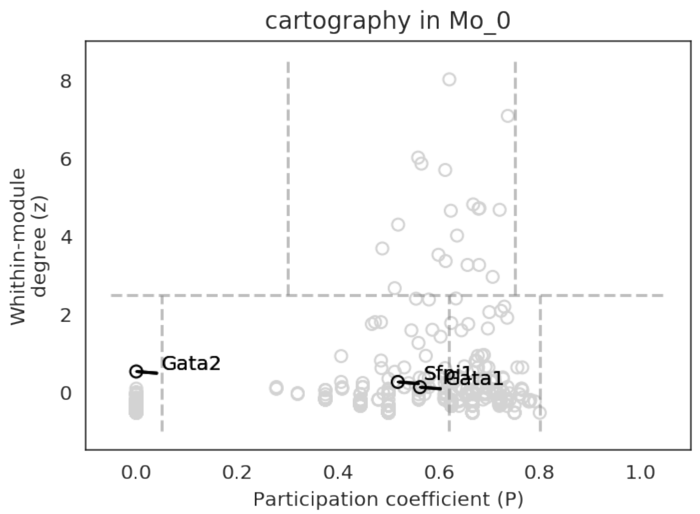
```
MEP_0
```


cartography in MEP_0
Gata2
Gata1
Sfpi1
Whithin-module degree (z)
Participation coefficient (P)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10

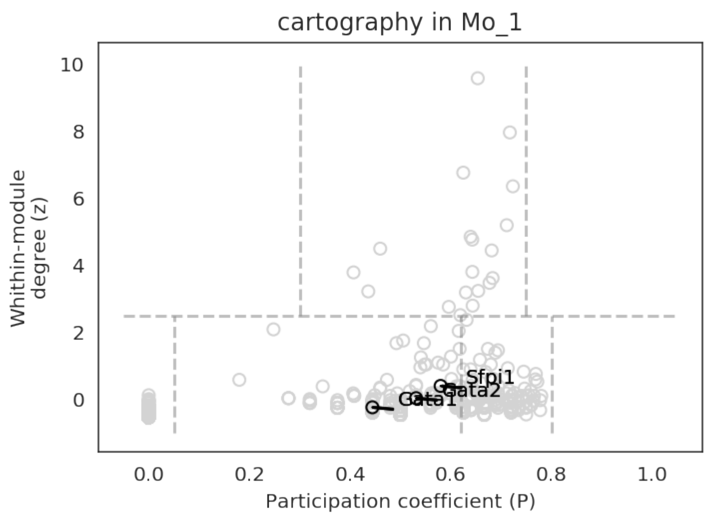
Mk_0


cartography in Mk_0
Gata2
Gata1
Sfpi1
Whithin-module degree (z)
Participation coefficient (P)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8

Mo_0

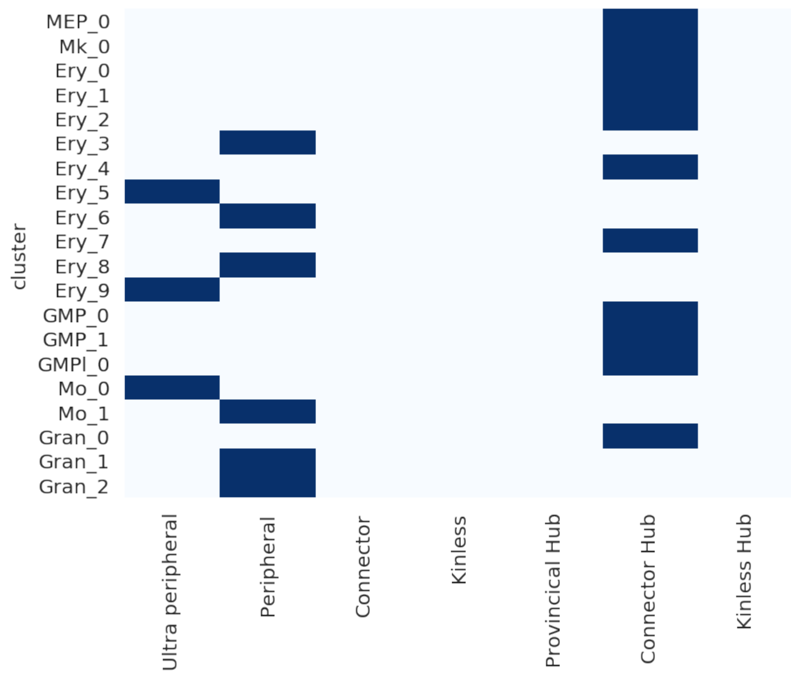



Mo_1




[66]:
```
# Plot the summary of cartography analysis
links.plot_cartography_term(goi="Gata2", save=f"{save_folder}/cartography")
```

```
Gata2
```



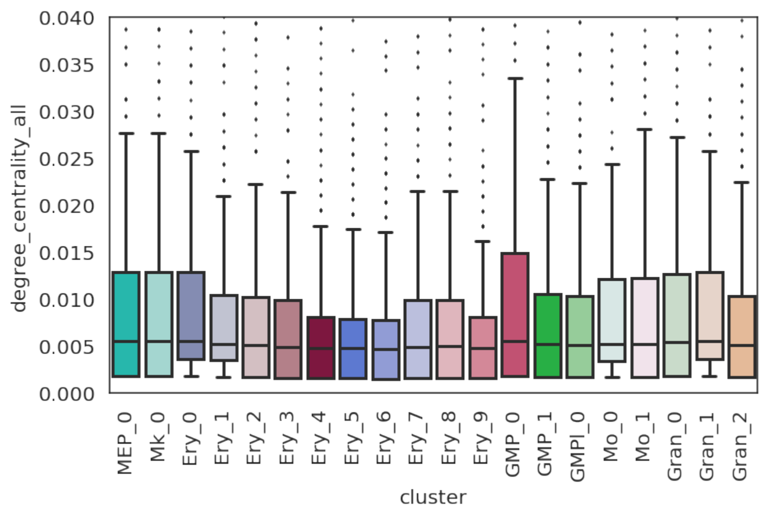

## 8. Network analysis; network score distribution

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_discributions(values=["degree_centrality_all"], method="boxplot",␣
↪save=f"{save_folder}")
```

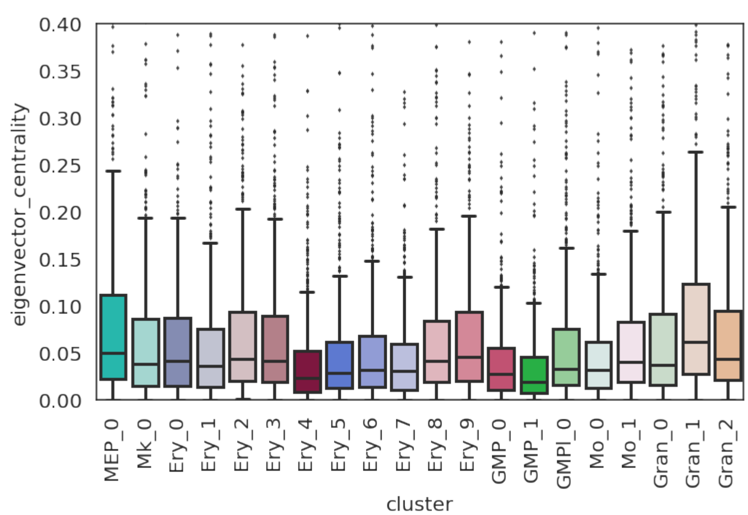
```
degree_centrality_all
```




[61]:

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

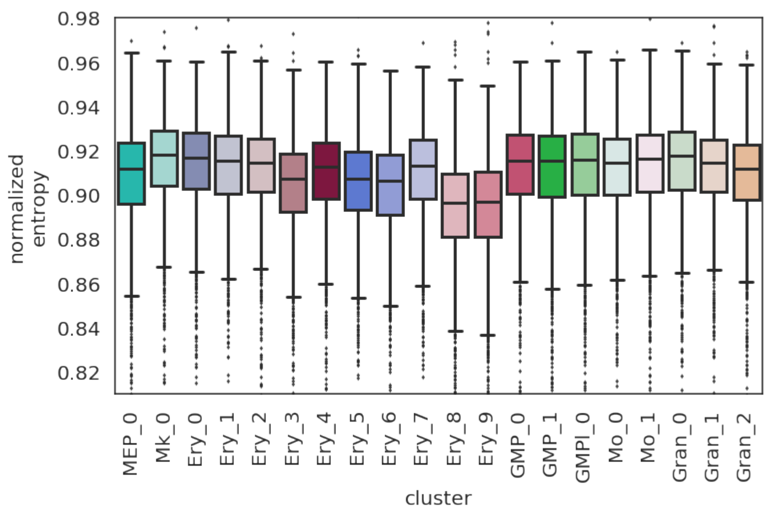
```
eigenvector_centrality
```



### 8.2. Distribution of netowrk 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.

Additonally, 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 determines 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 specicif 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
```

(continues on next page)

(continued from previous page)

```
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.

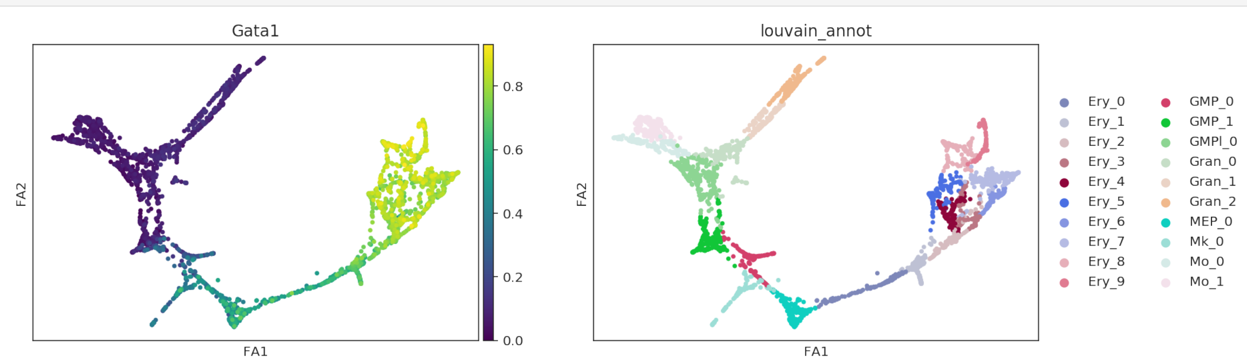
In this notebook, we'll show an example of the simulation; we'll simulate knock-out of Gata1 gene in the hematopoiesis.

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.

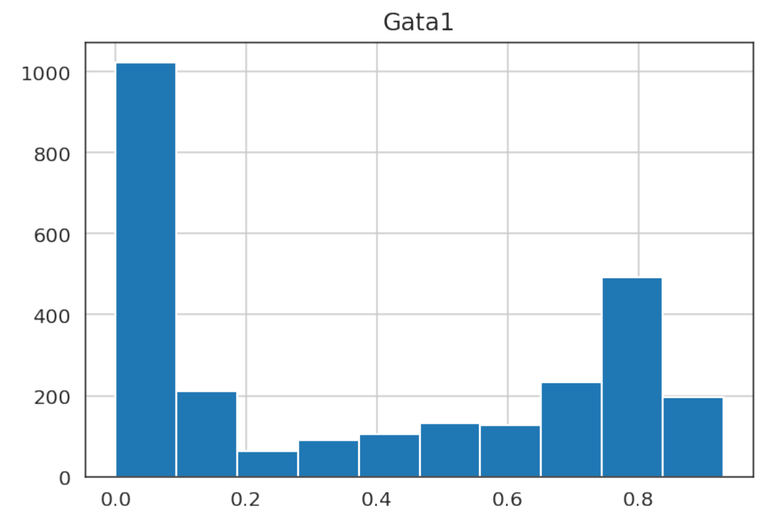
Here, we will analyze Gata1 for the demonstration of celloracle; Celloracle try to recapitulate the previous findings of Gata1 gene above.

#### 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 itelative calculations of signal propagations within the GRN.

Next step, we will calculate the probabilityof 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 trjectory graph.

(2) Markof simulation.

In Step 4.2 and 4.3, we usefunctions 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

[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)
```

(continues on next page)

(continued from previous page)

```
# 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

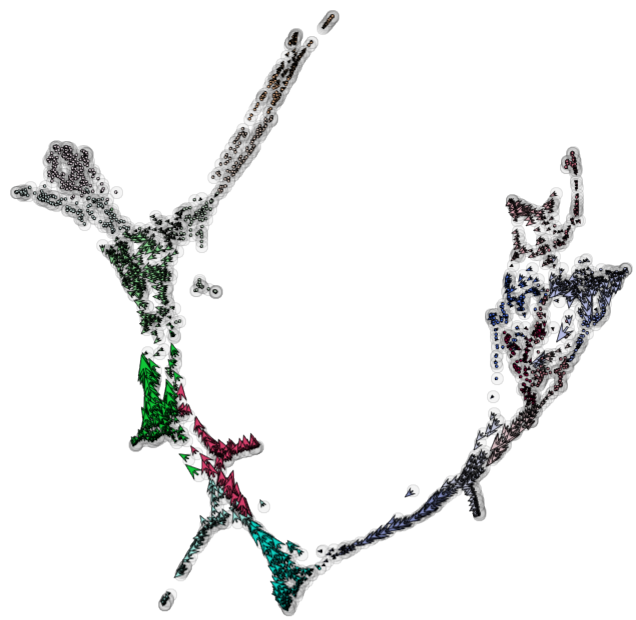
### 4.1. Detailed directed trajectory graph

[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_kwargs=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_kwargs)

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

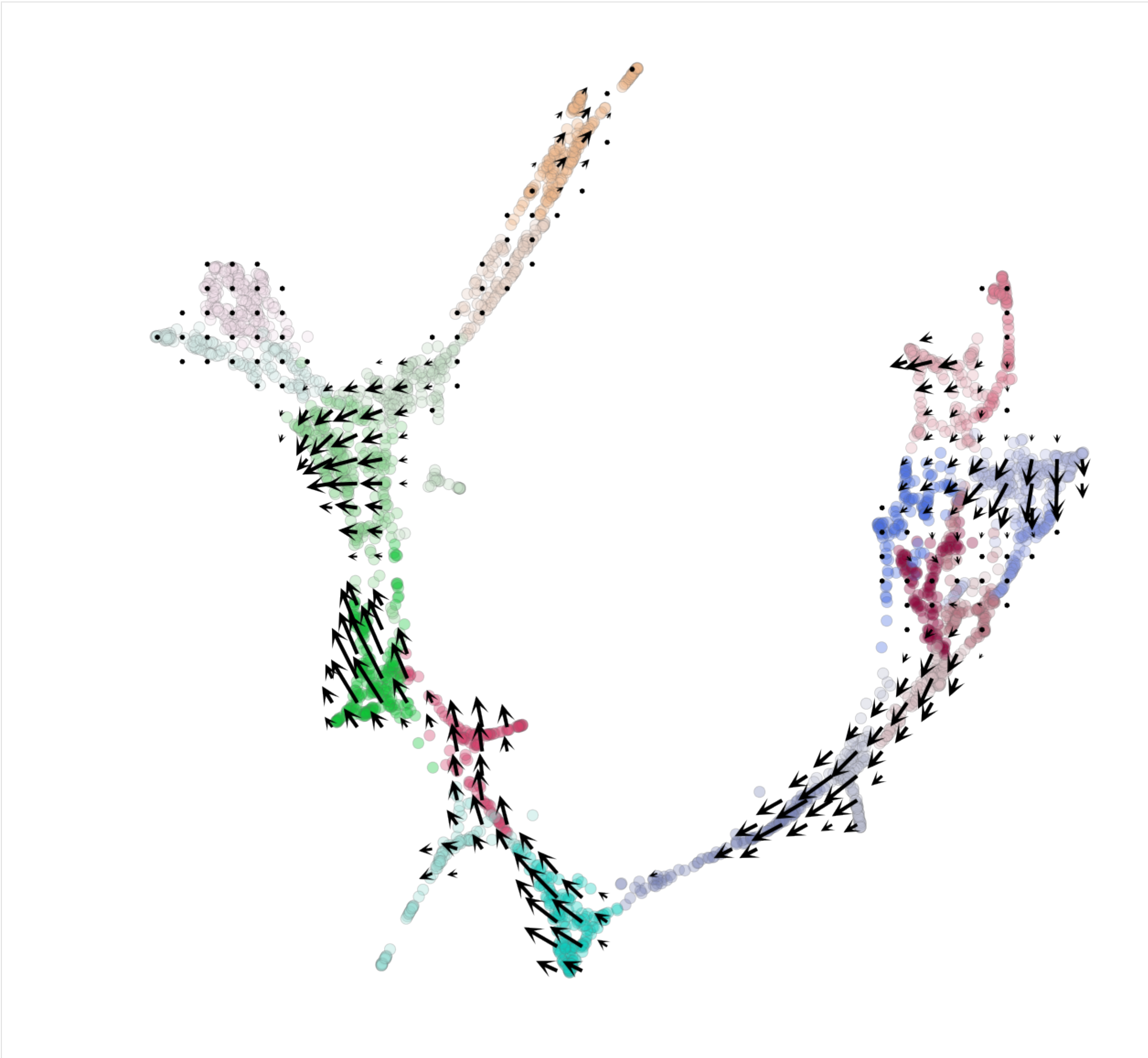
[36]:

```
(-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_kwargs_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 Markof 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
```

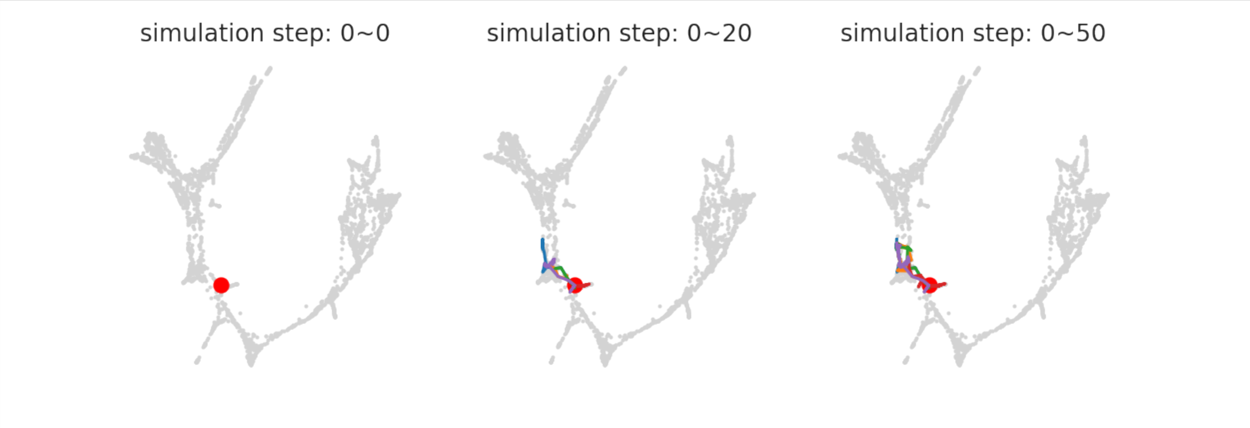
### 5.2. Check the results of the simulation for specific cells

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
```



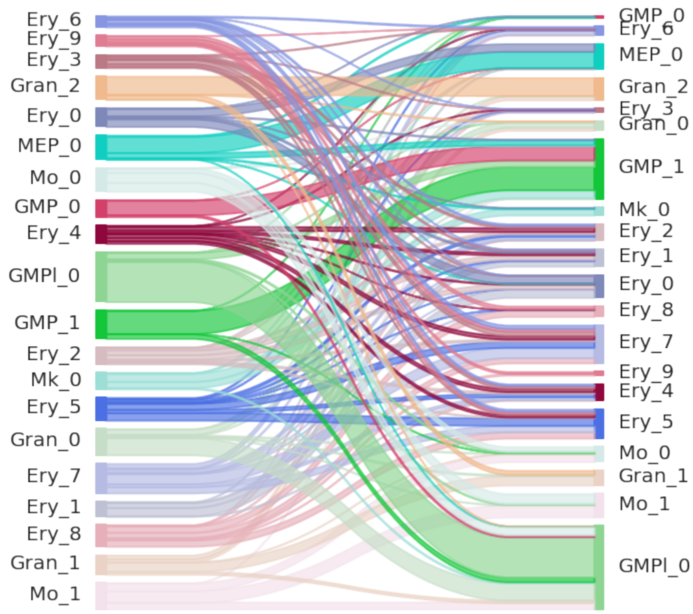

```
cell 1567
```


### 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_resutls_as_sankey(cluster_use=cl, start=0, end=100)
```

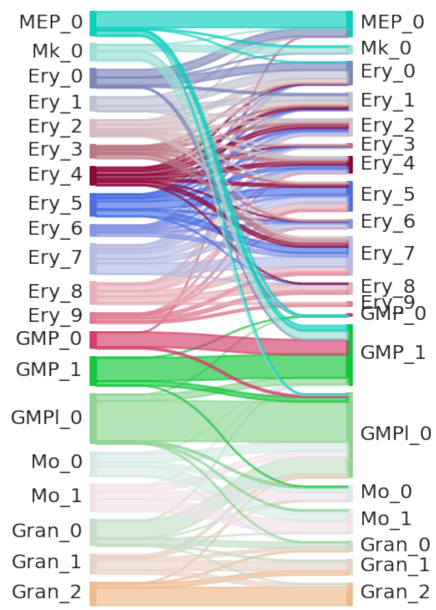



The Sankey diagram above looks messy because the cluster order is random.

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

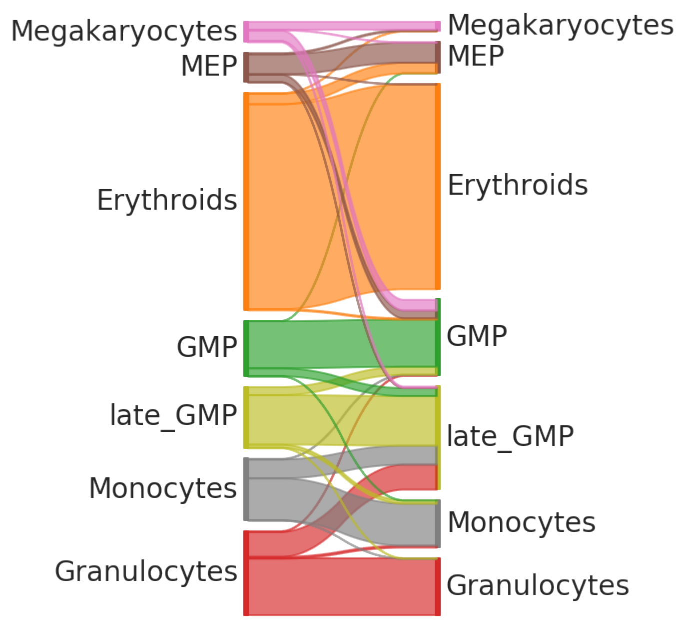
[90]:

```
cl = "louvain_annot"
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', 'GMPl_0', 'GMPl_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_resutls_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_resutls_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 sores 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 srt*) – 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 srt*) – 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_discributions**(*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 wich 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*, *cellstate=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_standerdized**
Almost the same as gem, but the gene_expression_matrix was standardized.

**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-exiting 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) VelycytoLoom: 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 cordinates

**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. Chose 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. Chose 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.pyplit.plot. See matplotlib documentation for details (https://matplotlib.org/api/_as_gen/matplotlib.pyplot.plot.html#matplotlib.pyplot.plot).

**plot_mc_resutls_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*, *calculate_randomized=True*)
Do Markov simlations 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 sumarized 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**()
Deepcoty itself.

**filter_motifs_by_score**(*threshold*, *method='cumlative_score'*)
Remove motifs with low binding scores.

**Parameters** **method** (*str*) – thresholding method. Select either of ["indivisual_score", "cumlative_score"]

**filter_peaks**(*peaks_to_be_remained*)
Filter peaks.

**Parameters** **peaks_to_be_remained** (*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 stat 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 sores 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 srt*) – 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 srt*) – 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_discributions** (*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 wich 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 objenct.

**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*, *return_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 transfered. 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 transfered. 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 validataion 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** (*padas.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*, *cluster_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

- *0.5.1 <2020-08-4>*

  - Add new promoter-TSS reference data for several reference genomes;(1)"Xenopus": ["xenTro2", "xenTro3"],(2)"Rat": ["rn4", "rn5", "rn6"], (3)"Drosophila": ["dm3", "dm6"], (4)"C.elegans": ["ce6", "ce10"], (5)"Arabidopsis": ["tair10"].

  - Add new motif data for several species: "Xenopus", "Rat", "Drosophila", "C.elegans" and "Arabidopsis".

- *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 genomepy (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

## 1.5 License

"Legal Entity" shall mean the union of the acting entity and all other entities that control, are controlled by, or are under common control with that entity. For the purposes of this definition, "control" means (i) the power, direct or indirect, to cause the direction or management of such entity, whether by contract or otherwise, or (ii) ownership of fifty percent (50%) or more of the outstanding shares, or (iii) beneficial ownership of such entity.

"You" (or "Your") shall mean an individual or Legal Entity exercising permissions granted by this License.

"Source" form shall mean the preferred form for making modifications, including but not limited to software source code, documentation source, and configuration files.

"Object" form shall mean any form resulting from mechanical transformation or translation of a Source form, including but not limited to compiled object code, generated documentation, and conversions to other media types.

"Work" shall mean the work of authorship, whether in Source or Object form, made available under the License, as indicated by a copyright notice that is included in or attached to the work (an example is provided in the Appendix below).

"Derivative Works" shall mean any work, whether in Source or Object form, that is based on (or derived from) the Work and for which the editorial revisions, annotations, elaborations, or other modifications represent, as a whole, an original work of authorship. For the purposes of this License, Derivative Works shall not include works that remain separable from, or merely link (or bind by name) to the interfaces of, the Work and Derivative Works thereof.

"Contribution" shall mean any work of authorship, including the original version of the Work and any modifications or additions to that Work or Derivative Works thereof, that is intentionally submitted to Licensor for inclusion in the Work by the copyright owner or by an individual or Legal Entity authorized to submit on behalf of the copyright owner. For the purposes of this definition, "submitted" means any form of electronic, verbal, or written communication sent to the Licensor or its representatives, including but not limited to communication on electronic mailing lists, source code control systems, and issue tracking systems that are managed by, or on behalf of, the Licensor for the purpose of discussing and improving the Work, but excluding communication that is conspicuously marked or otherwise designated in writing by the copyright owner as "Not a Contribution."

"Contributor" shall mean Licensor and any individual or Legal Entity on behalf of whom a Contribution has been received by Licensor and subsequently incorporated within the Work.

2. Grant of Copyright License. Subject to the terms and conditions of this License, each Contributor hereby grants to You a perpetual, worldwide, non-exclusive, no-charge, royalty-free, irrevocable copyright license to reproduce, prepare Derivative Works of, publicly display, publicly perform, sublicense, and distribute the Work and such Derivative Works in Source or Object form.

3. Grant of Patent License. Subject to the terms and conditions of this License, each Contributor hereby grants to You a perpetual, worldwide, non-exclusive, no-charge, royalty-free, irrevocable (except as stated in this section) patent license to make, have made, use, offer to sell, sell, import, and otherwise transfer the Work, where such license applies only to those patent claims licensable by such Contributor that are necessarily infringed by their Contribution(s) alone or by combination of their Contribution(s) with the Work to which such Contribution(s) was submitted. If You institute patent litigation against any entity (including a cross-claim or counterclaim in a lawsuit) alleging that the Work or a Contribution incorporated within the Work constitutes direct or contributory patent infringement, then any patent licenses granted to You under this License for that Work shall terminate as of the date such litigation is filed.

4. Redistribution. You may reproduce and distribute copies of the Work or Derivative Works thereof in any medium, with or without modifications, and in Source or Object form, provided that You meet the following conditions:

(a) You must give any other recipients of the Work or Derivative Works a copy of this License; and

(b) You must cause any modified files to carry prominent notices stating that You changed the files; and

(c) You must retain, in the Source form of any Derivative Works that You distribute, all copyright, patent, trademark, and attribution notices from the Source form of the Work, excluding those notices that do not pertain to any part of the Derivative Works; and

(d) If the Work includes a "NOTICE" text file as part of its distribution, then any Derivative Works that You distribute must include a readable copy of the attribution notices contained within such NOTICE file, excluding those notices that do not pertain to any part of the Derivative Works, in at least one of the following places: within a NOTICE text file distributed as part of the Derivative Works; within the Source form or documentation, if provided along with the Derivative Works; or, within a display generated by the Derivative Works, if and wherever such third-party notices normally appear. The contents of the NOTICE file are for informational purposes only and do not modify the License. You may add Your own attribution notices within Derivative Works that You distribute, alongside or as an addendum to the NOTICE text from the Work, provided that such additional attribution notices cannot be construed as modifying the License.

You may add Your own copyright statement to Your modifications and may provide additional or different license terms and conditions for use, reproduction, or distribution of Your modifications, or for any such Derivative Works as a whole, provided Your use, reproduction, and distribution of the Work otherwise complies with the conditions stated in this License.

5. Submission of Contributions. Unless You explicitly state otherwise, any Contribution intentionally submitted for inclusion in the Work by You to the Licensor shall be under the terms and conditions of this License, without any additional terms or conditions. Notwithstanding the above, nothing herein shall supersede or modify the terms of any separate license agreement you may have executed with Licensor regarding such Contributions.

6. Trademarks. This License does not grant permission to use the trade names, trademarks, service marks, or product names of the Licensor, except as required for reasonable and customary use in describing the origin of the Work and reproducing the content of the NOTICE file.

7. Disclaimer of Warranty. Unless required by applicable law or agreed to in writing, Licensor provides the Work (and each Contributor provides its Contributions) on an "AS IS" BASIS, WITHOUT WARRANTIES OR CONDITIONS OF ANY KIND, either express or implied, including, without limitation, any warranties or conditions of TITLE, NON-INFRINGEMENT, MERCHANTABILITY, or FITNESS FOR A PARTICULAR PURPOSE. You are solely responsible for determining the appropriateness of using or redistributing the Work and assume any risks associated with Your exercise of permissions under this License.

8. Limitation of Liability. In no event and under no legal theory, whether in tort (including negligence), contract, or otherwise, unless required by applicable law (such as deliberate and grossly negligent acts) or agreed to in writing, shall any Contributor be liable to You for damages, including any direct, indirect, special, incidental, or consequential damages of any character arising as a result of this License or out of the use or inability to use the Work (including but not limited to damages for loss of goodwill, work stoppage, computer failure or malfunction, or any and all other commercial damages or losses), even if such Contributor has been advised of the possibility of such damages.

9. Accepting Warranty or Additional Liability. While redistributing the Work or Derivative Works thereof, You may choose to offer, and charge a fee for, acceptance of support, warranty, indemnity, or other liability obligations and/or rights consistent with this License. However, in accepting such obligations, You may act only on Your own behalf and on Your sole responsibility, not on behalf of any other Contributor, and only if You agree to indemnify, defend, and hold each Contributor harmless for any liability incurred by, or claims asserted against, such Contributor by reason of your accepting any such warranty or additional liability.

END OF TERMS AND CONDITIONS

APPENDIX: How to apply the Apache License to your work.

To apply the Apache License to your work, attach the following boilerplate notice, with the fields enclosed by brackets "[]" replaced with your own identifying information. (Don't include the brackets!) The text should be enclosed in the appropriate comment syntax for the file format. We also recommend that a file or class name and description of purpose be included on the same "printed page" as the copyright notice for easier identification within third-party archives.

## 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.

CHAPTER

TWO

# INDICES AND TABLES

- genindex
- modindex
- search

# PYTHON MODULE INDEX

## C

# INDEX

## R



## S



## T



## U