celloracle

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

Source code are available at celloracle git hub repository

Note:

For colleagues in Morris Lab
Thank you for your kind help!!

Documentation is also available as a word and pdf file.

`pdf documentation`

`word documentation` The word documentation does not include figures.

Could you please add comments on the file and send it back to Kenji?
I really appreciate your support!

Thanks,
Kenji

(This message will be deleted when we launch celloracle.)

Warning: CellOracle is still under development. It is alpha 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 Python Requirements

- `celloracle` was developed with python 3.6. We do not support python 2.7x or python <=3.5.
- `celloracle` was developed in Linux and macOS. We do not guarantee that `celloracle` works in Windows OS.
- If you want to use `celloracle` in Windows OS, we recommend using Windows Subsystem for Linux to built environment. In that case, please select Ubuntu18.04 LTS.
- We highly recommend using anaconda to setup python environment.
- 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 below 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 . On Mac OS, you may have a compile error during velocyto installation. I recommend you installing Xcode in that case.

```
conda install numpy scipy cython numba matplotlib scikit-learn h5py click
```

Then

```
pip install velocyto
```

3. Install scanpy

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

```
conda install seaborn scikit-learn statsmodels numba pytables python-igraph louvain
```

Then

```
pip install scanpy
```

4. Install gimmemotifs

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

```
conda install gimmemotifs genomepy=0.5.5
```

5. Install other python libraries

Please install other python libraries below with the following commands.

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

6. install celloracle from github

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

1.1.2 R requirements

`celloracle` use R library 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` with the following r-script or the author's instruction . If you have no plan for scATAC-seq analysis and just want to use `celloracle` with a default TF information which was supplied with celloracle, you can skip installation of `Cicero`.

in R console,

```
if (!requireNamespace("BiocManager", quietly = TRUE))
install.packages("BiocManager")
BiocManager::install("cicero", version = "3.8")
```

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

`rnetcarto` installation has to be done with several steps. Please install rnetcarto with the author's instruction . You need to install the GNU Scientific Libraries before installing rnetcarto. Detailed instruction can be found here .

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.

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

1.2 Tutorial

The analysis proceeds through multiple steps. Please run the notebooks following the number of the notebook. 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 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 get open accessible promoter/enhancer DNA sequence. We can get active proximal promoter/enhancer genome sequence by picking up 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 co-access score and TSS information.

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

A. Extract TF binding information from scATAC-seq data

If you have a scATAC-seq data, you can get information of distal cis-regulatory elements. This step uses Cicero and does not use celloracle. Please refer to the documentation of Cicero for the detailed usage.

R notebook

0. Import library

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

1. Prepare data

In this tutorial we use acATAC-seq data in 10x genomics database. You do not need to download these data if you analyze your scATAC 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"))
```

(continues on next page)

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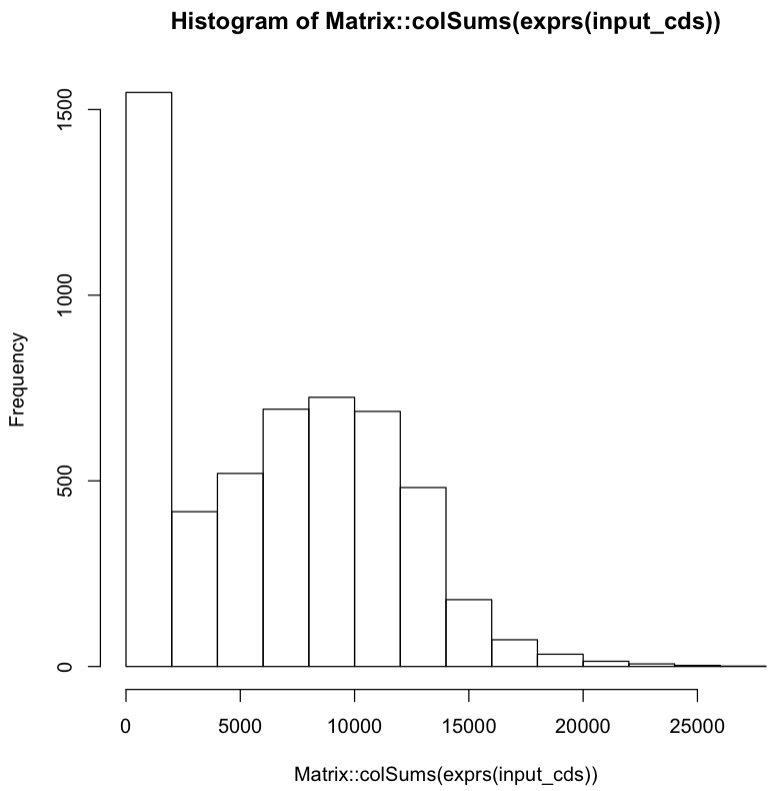
```
row.names(cellinfo) <- cellinfo$V1
names(cellinfo) <- "cells"

# 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(newCellDataSet(indata,
                           phenoData = methods::new("AnnotatedDataFrame", data =␣
↪cellinfo),
                           featureData = methods::new("AnnotatedDataFrame", data =␣
↪peakinfo),
                           expressionFamily=VGAM::binomialff(),
                           lowerDetectionLimit=0))
input_cds@expressionFamily@vfamily <- "binomialff"
input_cds <- monocle::detectGenes(input_cds)

#Ensure there are no peaks included with zero reads
input_cds <- input_cds[Matrix::rowSums(exprs(input_cds)) >= 100,]
```

3. Qauality check and Filtering

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



[9]:
```
# filter cells by peak_count
max_count <-  15000 # Please change the threshold value according to the distribution␣
↪of the peak_count of your data
min_count <- 2000 # Please change the threshold value according to the distribution␣
↪of the peak_count of your data
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

[10]:
```
# Run cicero to get cis-regulatory networks
set.seed(2017)
input_cds <- detectGenes(input_cds)
input_cds <- estimateSizeFactors(input_cds)

input_cds <- reduceDimension(input_cds, max_components = 2, verbose=T,scaling = FALSE,
↪relative_expr=FALSE,
                      reduction_method = 'tSNE', norm_method = "none")

tsne_coords <- t(reducedDimA(input_cds))
row.names(tsne_coords) <- row.names(pData(input_cds))
cicero_cds <- make_cicero_cds(input_cds, reduced_coordinates = tsne_coords)

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

```
Remove noise by PCA ...

Reduce dimension by tSNE ...

Overlap QC metrics:
Cells per bin: 50
Maximum shared cells bin-bin: 44
Mean shared cells bin-bin: 0.76256263875674
Median shared cells bin-bin: 0
```

5. Run cicero to get cis-regulatory connection scores

[11]:
```
# import genome length, which is needed for the function, run_cicero
mm10_chromosome_length <- read.table("./mm10_chromosome_length.txt")

# runc the main function
conns <- run_cicero(cicero_cds, mm10_chromosome_length) # Takes a few minutes to run

# check results
head(conns)
```

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

Next, the results of Cicero analysis will be processed to make TSS annotations.

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 time

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

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

!! Please make sure that you are setting correct reference genoms.

[5]:
```
tss_annotated = ma.get_tss_info(peak_str_list=peaks, ref_genome="mm10")

# 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 of TSS peak or peaks that have a connection with a TSS peak. "gene_short_name" is a gene name of the TSS site. "coaccess" is a co-access score between a peak and TSS peak. If the score is 1, it means that the peak is TSS itself.

[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 open accessible promoter/enhancer sequence.

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 time

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 bed file of ATAC-seq data. This script can also be used 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

!! Please make sure that you are setting correct ref genoms.

[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 open-accessible Promoter/enhancer DNAs using ATAC-seq data. Next, we scan the regulatory genomic sequence searching for the TF-binding motifs to get the list of TFs for each target gene. In the later GRN inference process, this TF list per target gene will be used as a potential regulatory connection.

Python notebook

0. Import libraries

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

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```
import seaborn as sns
#import time

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

%config InlineBackend.figure_format = 'retina'

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

[3]:
```
from celloracle import motif_analysis as ma
from celloracle.utility import save_as_pickled_object
```

1. Load data

load annotated peak data.

[4]:
```
peaks = pd.read_parquet("../01_ATAC-seq_data_processing/option1_scATAC-seq_data_
↪analysis_with_cicero/peak_file.parquet")
peaks.head()
```

[4]:

	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. Check data

[5]:
```
# check data
print(f"number of peak: {len(peaks.peak_id.unique())}")
mean_ = len(peaks.groupby(["gene_short_name", "peak_id"]).count())/len(peaks.gene_
↪short_name.unique())
print(f"mean peaks per gene: {mean_}")

def getLength(x):
    a, b, c = x["peak_id"].split("_")
    return int(c) - int(b)

#
df = peaks.apply(lambda x: getLength(x), axis=1)
print(f"mean peak length: {df.values.mean()}")
```

```
number of peak: 13919
mean peaks per gene: 1.0591731964333964
mean peak length: 1756.1744260204082
```

2.1. Remove short peaks

Short DNA fragment, such as less than 5 bases, cannot be used for motif scan. Remove short DNA fragment.

[6]:
```
peaks = peaks[df>=5]
```

3. Instantiate TFinfo object and search for TF binding motifs

The motif analysis module has a custom class; TFinfo. TFinfo object converts a peak data into a DNA sequence and scans the DNA sequence searching for TF binding motifs. Then the results of motif scan will be filtered and converted into several files, such as python dictionary or dataframe. This TF information is necessary for GRN inference.

3.1 check reference genome installation

[7]:
```
# PLEASE make sure that you are setting correct ref genome.
ref_genome = "mm10"

ma.is_genome_installed(ref_genome=ref_genome)
```

```
genome mm10 is not installed in this environment.
Please install genome using genomepy.
e.g.
    >> import genomepy
    >> genomepy.install_genome("mm9", "UCSC")
```

[7]:
```
False
```

3.2. Install reference genome (if refgenome is not installed)

[9]:
```
import genomepy
genomepy.install_genome(ref_genome, "UCSC")
```

```
downloading from http://hgdownload.soe.ucsc.edu/goldenPath/mm10/bigZips/chromFa.tar.
↪gz...
done...
name: mm10
local name: mm10
fasta: /home/k/.local/share/genomes/mm10/mm10.fa
```

[9]:
```
# check again
ma.is_genome_installed(ref_genome=ref_genome)
```

[9]:
```
True
```

[14]:
```
# Instantiate TFinfo object
tfi = ma.TFinfo(peak_data_frame=peaks, # peak info calculated from ATAC-seq data
                ref_genome=ref_genome)
```

4. Scan motifs and save object

This step requires computational resource and may take long time

[15]:
```
%%time
# Scan motifs
tfi.scan(fpr=0.02, verbose=True)

# sace tfinfo object
tfi.to_hdf5(file_path="test.celloracle.tfinfo")
```

```
initiating scanner ...
```

```
2019-09-22 23:00:18,604 - INFO - Using background: genome mm10 with length 200
2019-09-22 23:00:18,986 - INFO - Determining FPR-based threshold
```

```
getting DNA sequences ...
scanning motifs ...
```

```
HBox(children=(IntProgress(value=1, bar_style='info', max=1), HTML(value='')))
```

```
CPU times: user 52min 23s, sys: 36.8 s, total: 53min
Wall time: 52min 58s
```

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

[16]:

	seqname	motif_id	factors_direct	factors_indirect	score	pos	strand
0	chr10_100015291_100017830	GM.5.0.Homeodomain.0001	TGIF1	ENSG00000234254, TGIF1	10.311002	1003	1
1	chr10_100015291_100017830	GM.5.0.Mixed.0001		SRF, EGR1	7.925873	481	1
2	chr10_100015291_100017830	GM.5.0.Mixed.0001		SRF, EGR1	7.321375	911	-1
3	chr10_100015291_100017830	GM.5.0.Mixed.0001		SRF, EGR1	7.276585	811	-1
4	chr10_100015291_100017830	GM.5.0.Nuclear_receptor.0002	NR2C2	NR2C2, Nr2c2	9.067331	449	-1

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

5. Filtering motifs

[17]:
```
# 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.
tfi.make_TFinfo_dataframe_and_dictionary(verbose=True)
```

```
peaks were filtered: 12934005 -> 2285279
1. converting scanned results into one-hot encoded dataframe.
```

```
HBox(children=(IntProgress(value=0, max=13919), HTML(value='')))
```

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

```
HBox(children=(IntProgress(value=0, max=14804), HTML(value='')))
```

```

```

```
HBox(children=(IntProgress(value=0, max=1090), HTML(value='')))
```

```

```

6. Get Final results

6.1. Get resutls as a dictionary

[18]:
```
td = tfi.to_dictionary(dictionary_type="targetgene2TFs")
```

6.2. Get results as a dataframe

[20]:
```
df = tfi.to_dataframe()
df.head()
```

[20]:

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

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

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

[5 rows x 1092 columns]

7. Save TFinfo as dictionary and dataframe

We'll use this information in the later step to make GRN. Save the results.

[21]:
```
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"))
```

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

- 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 of scanpy and the documentation of Seurat .

If you already have preprocessed scRNA-seq data, which includes 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 modification.

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's 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 reduces calculation time in the GRN reconstruction and simulation. We recommend using 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, differential gene calculation. However, we also need non-transformed gene expression data in the celloracle analysis. Thus we keep raw count in anndata with the following command before 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)
```

[8]:
```
# (optional) Regressing out some values help reduce batch effects.

#sc.pp.regress_out(adata, ['n_counts', 'percent_mito'])
#sc.pp.regress_out(adata, ['n_counts'])
```

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 of dimensional reduction so that the embedding appropriately represents the data structure. We recommend using one of the dimensional reduction algorithms (or trajectory inference algorithms); UMAP, tSNE, diffusion map, Force-directed graph drawing, 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

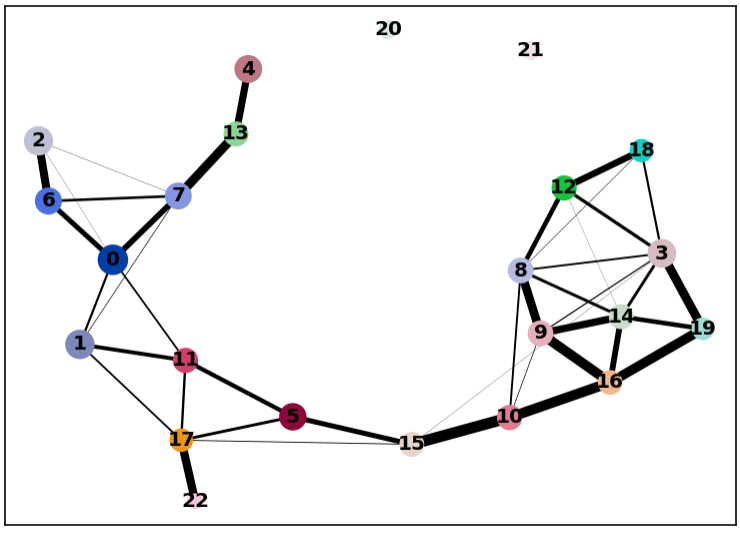
[12]:
```
# PAGA graph construction
sc.tl.paga(adata, groups='louvain')
```

[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]
```

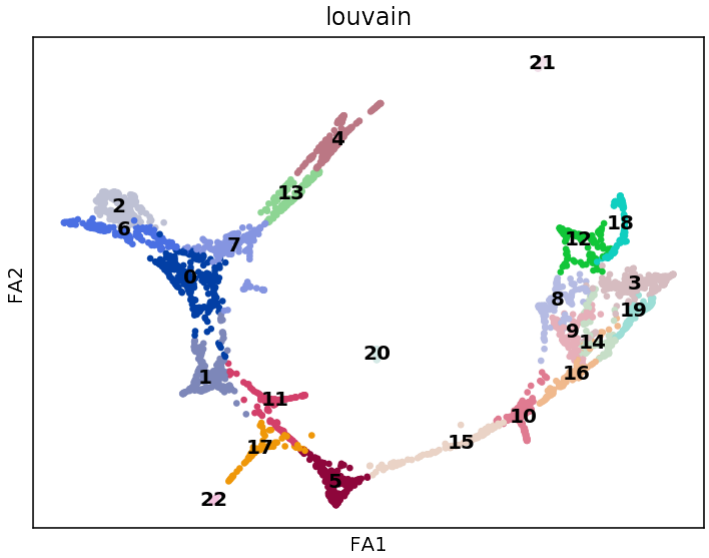
[14]:
```
plt.rcParams["figure.figsize"] = [6, 4.5]
```

[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')
```



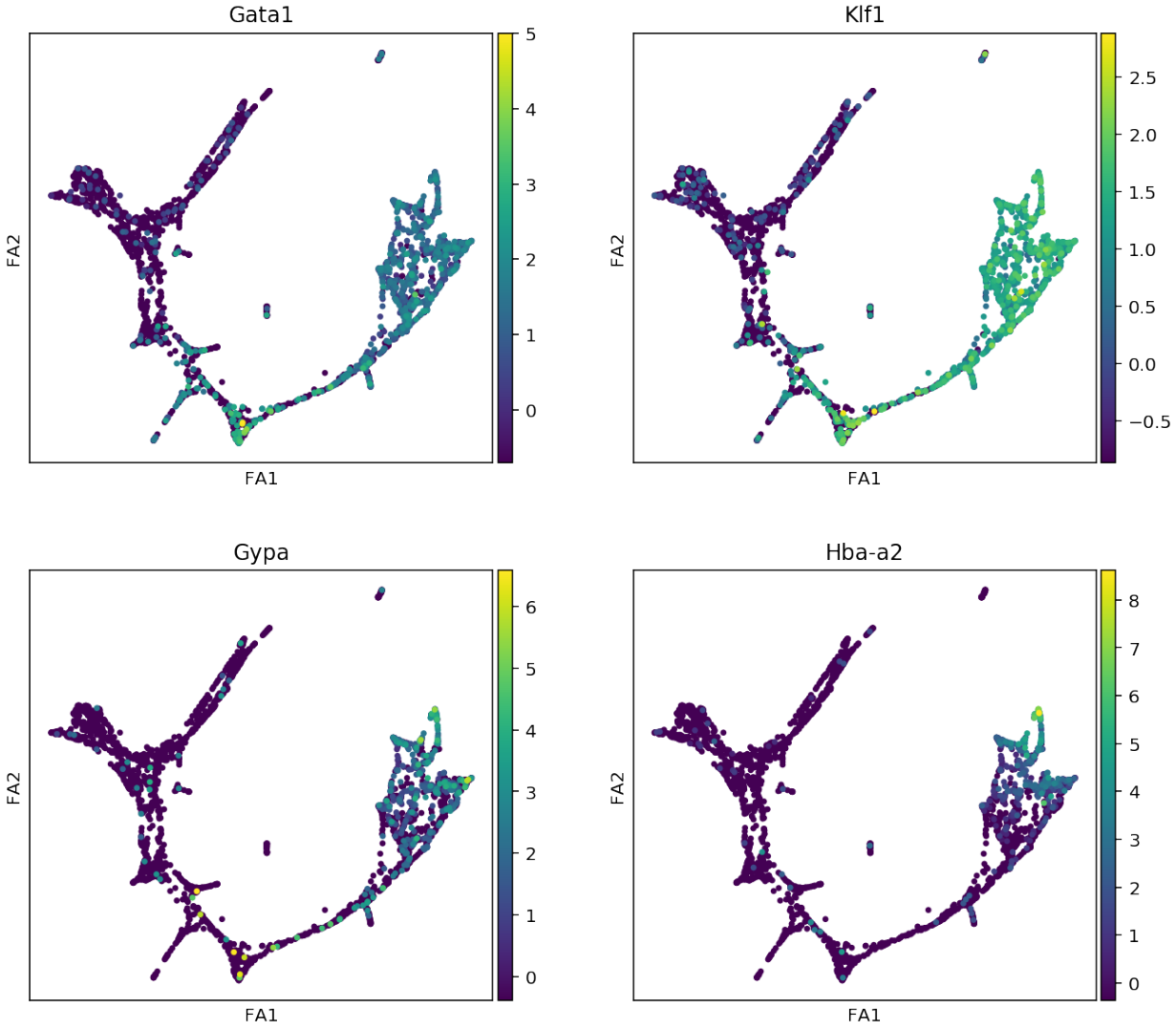
8. Check data

8.1. Visualize marker gene expression

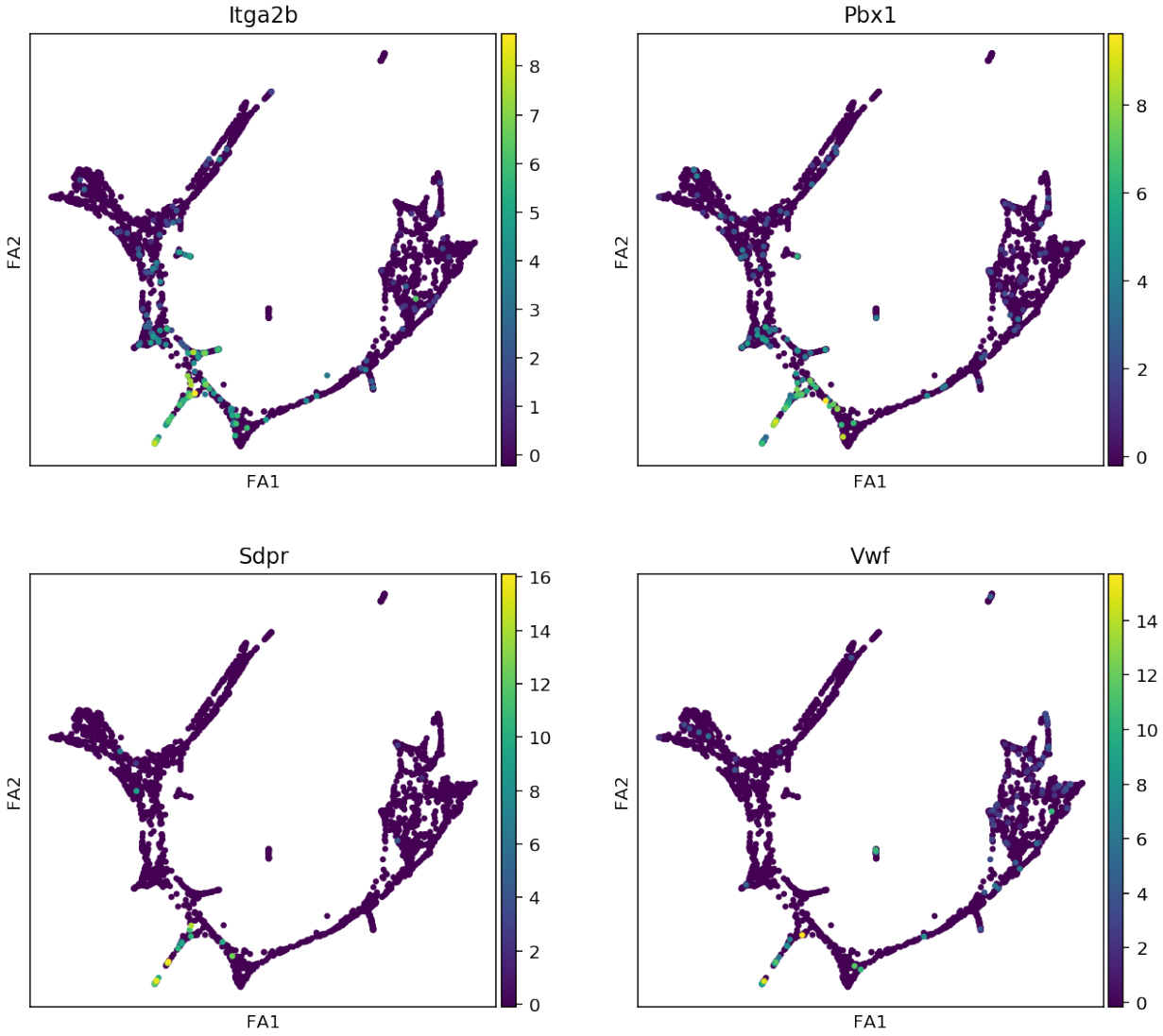
[18]:
```
plt.rcParams["figure.figsize"] = [4.5, 4.5]
```

[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()
```

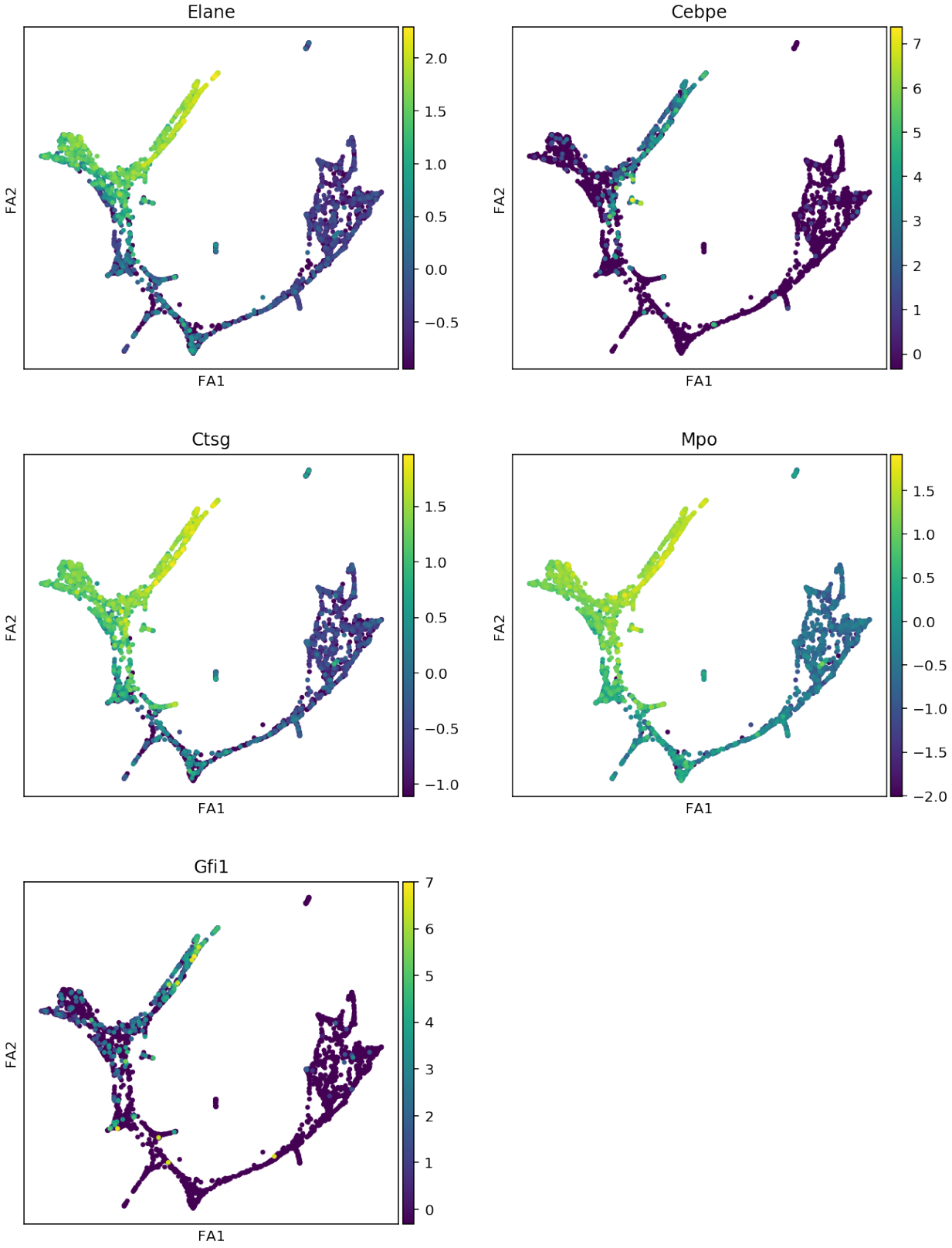
```
marker gene of Erythroids
```



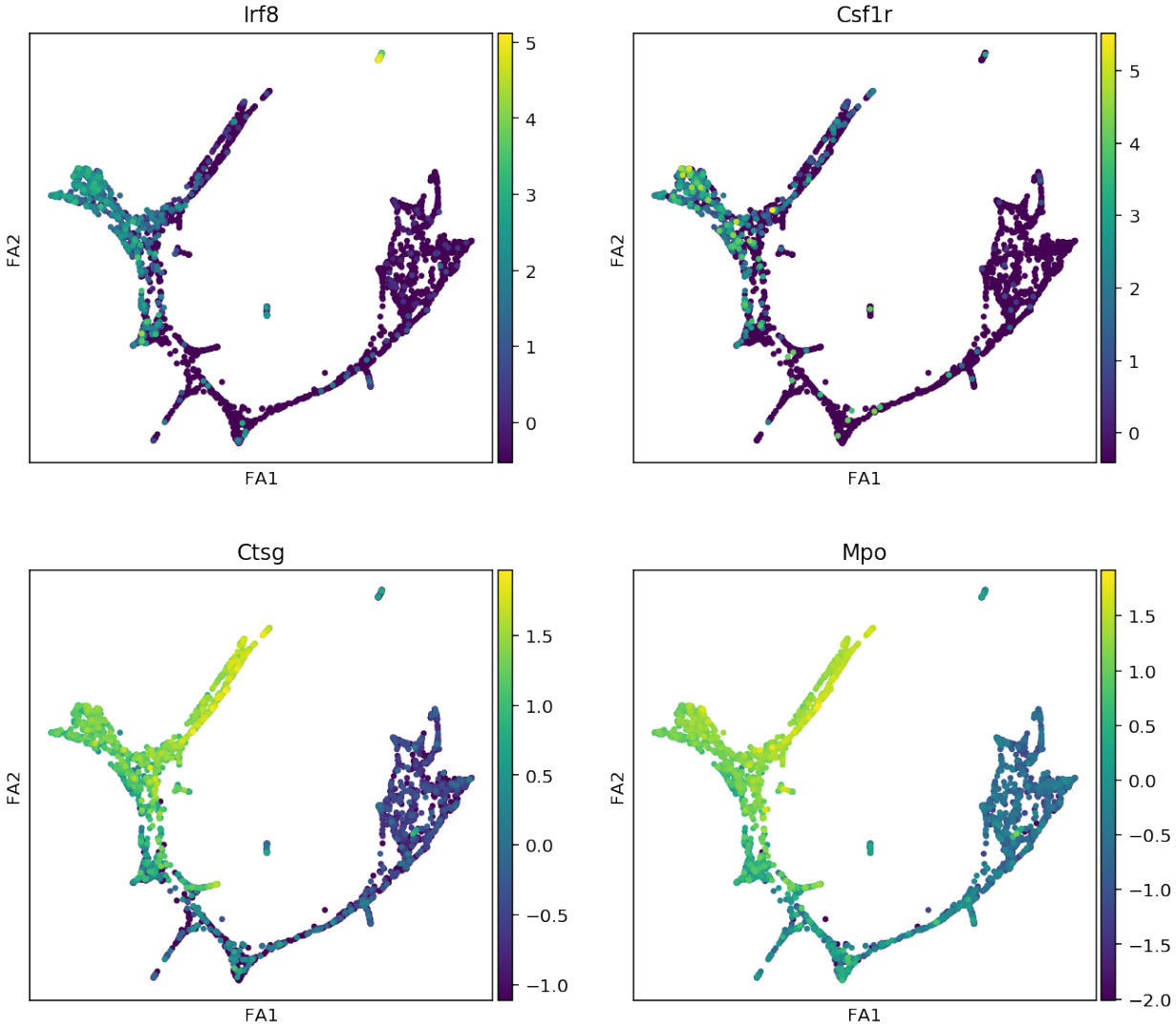

```
marker gene of Megakaryocytes
```



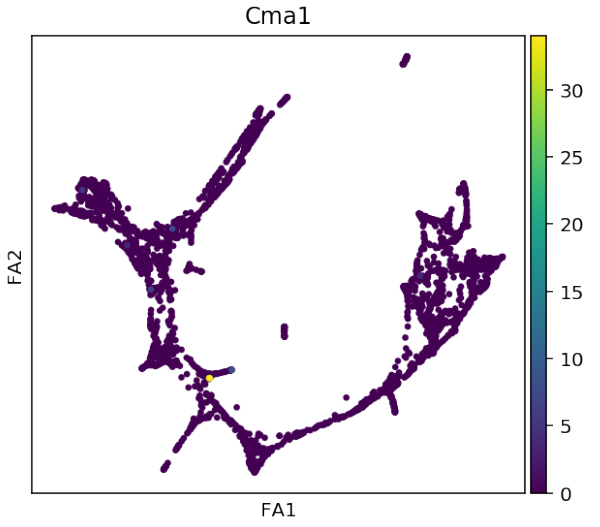

```
marker gene of Granulocytes
```

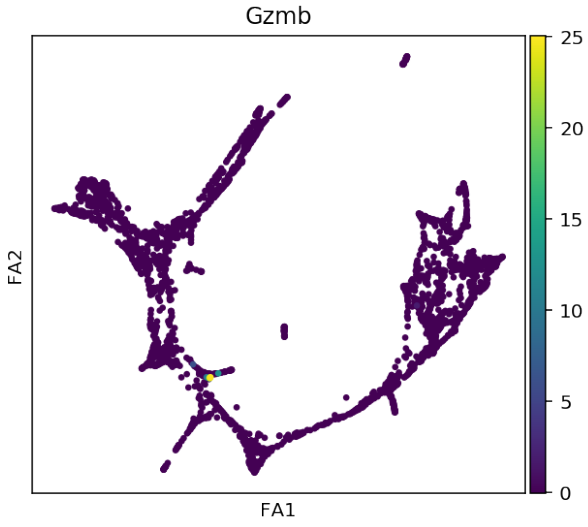


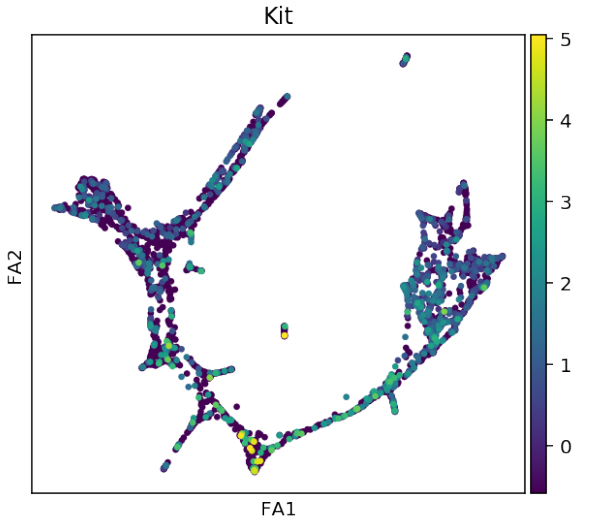

```
marker gene of Monocytes
```



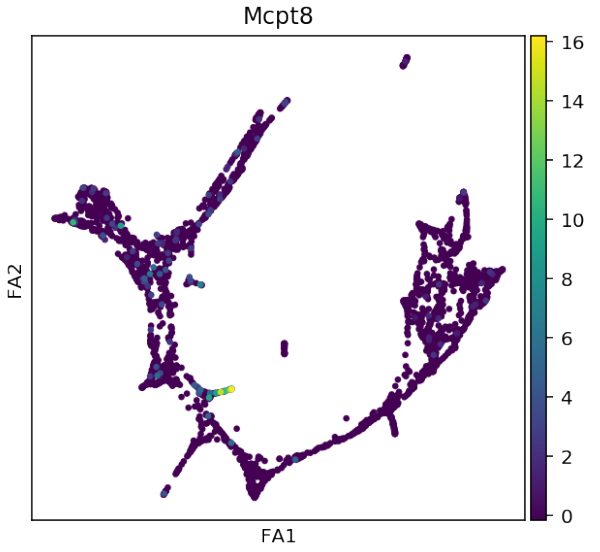

```
marker gene of Mast_cells
```

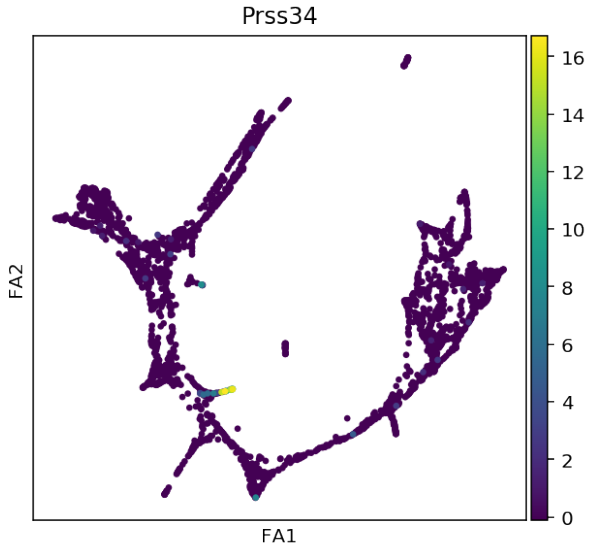







```
marker gene of Basophils
```



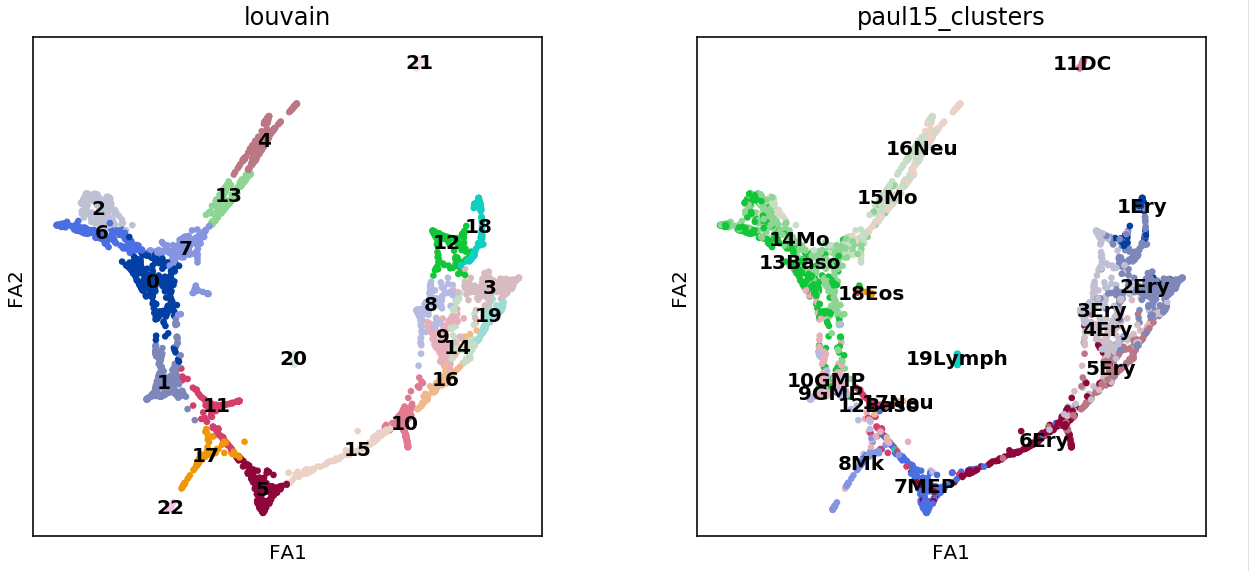


8. Make annotation for cluster

Based on the marker gene expression and previous report, we will manually make annotation for each cluster.

8.1. 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]
```

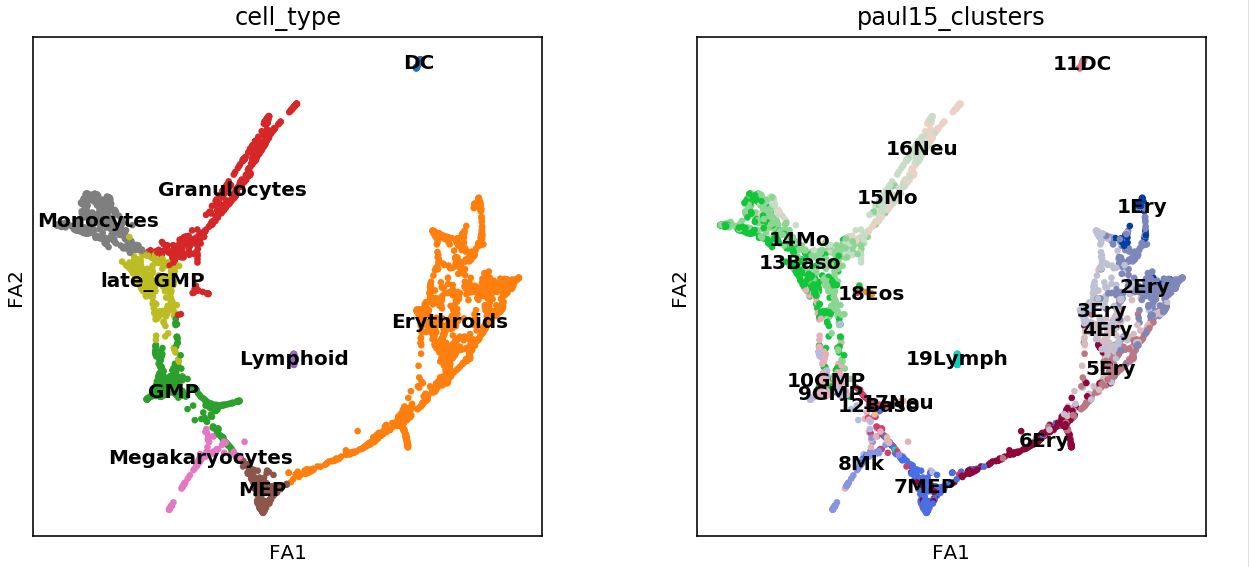
[22]:
```
# make anottation dictionary
annotation = {"MEP":[5],
              "Erythroids": [15, 10, 16, 9, 8, 14, 19, 3, 12, 18],
              "Megakaryocytes":[17, 22],
              "GMP":[11, 1],
              "late_GMP" :[0],
              "Granulocytes":[7, 13, 4],
              "Monocytes":[6,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

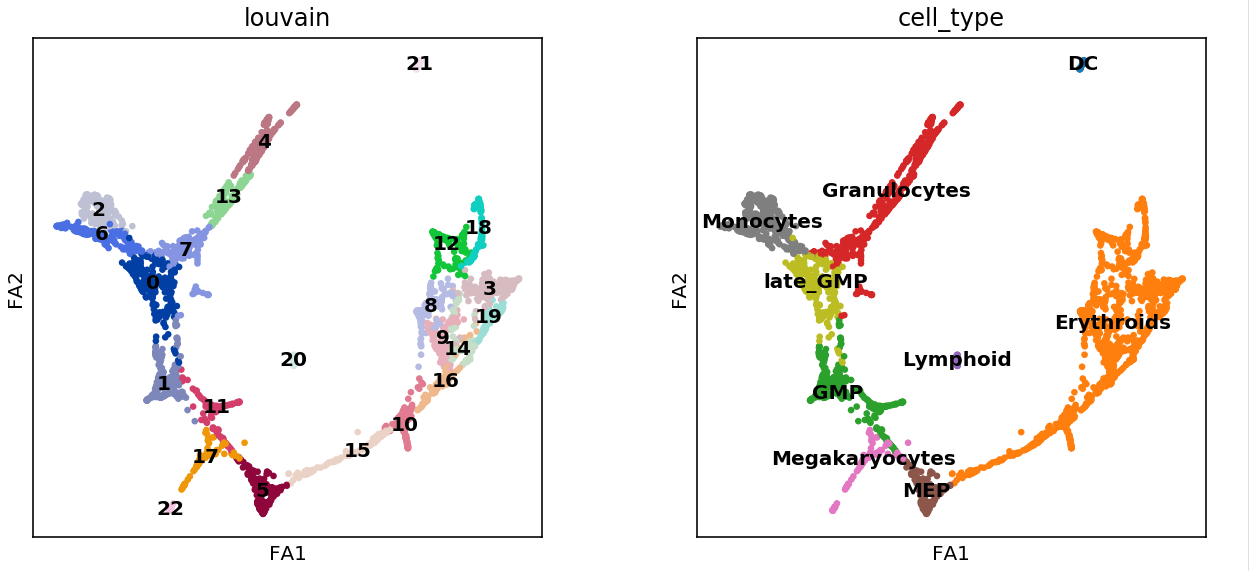
```
... storing 'cell_type' as categorical
```



8.2. 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')
```



[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',
```

(continues on next page)

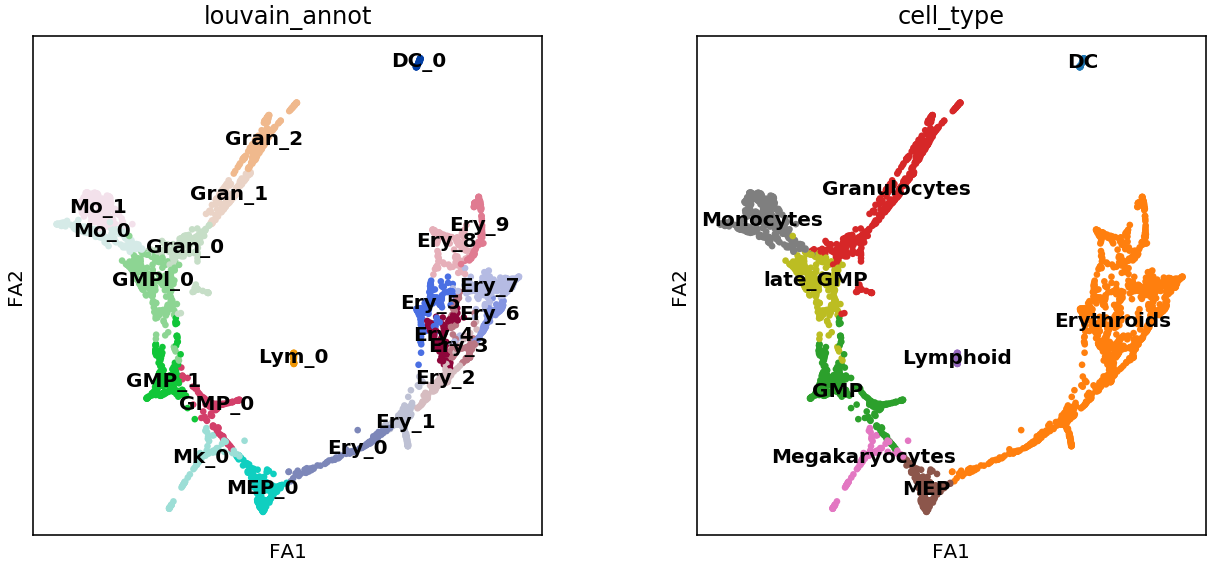
(continued from previous page)

```
                '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, dimensional reduction. In the next step, we'll do GRN inference, network analysis, and in silico simulation based on these information.

9. (Option) Subset cells

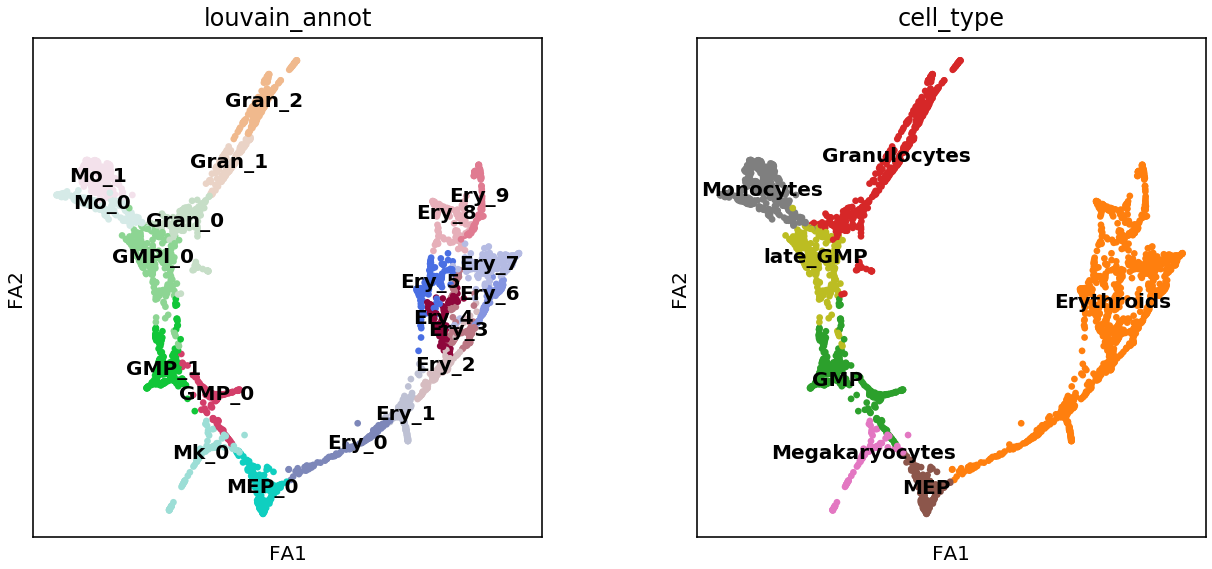
In this tutorial, we are using scRNA-seq data of hematopoiesis. In the latter part, we will focus on the cell fate decision in the myeloid lineage. So we remove non-myeloid cell cluster, DC and Lymphoid now.

[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')
```



10. Save data

[32]:
```
#adata.write_h5ad("data/Paul_etal_15.h5ad")
```

[ ]:
```

```

B. scRNA-seq data preprocessing with Seurat

R notebook . . . comming soon

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` have python API and command-line API to convert Seurat object into anndata. Please go to the documentation of celloracle API for more information.

1.2.4 Network analysis

`celloracle` import 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 GRN for each cluster enabling us to compare GRNs each other. It is also possible to analyze how the GRN change over differentiation. The dynamics of the GRN structure provide us insight into the context-dependent regulatory mechanism.

Python notebook

0. Import libraries

[6]:
```
# 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
```

[7]:
```
import celloracle as co
```

[8]:
```
# 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.

[5]:
```
co.network_analysis.test_R_libraries_installation()
```

```
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

[6]:
```
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.

[7]:
```
# 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")
```

1.2. Load TF data.

For the GRN inference, celloracle needs TF information, which contains lists of the regulatory candidate gene. 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 how to make TF information.

If you do not have your scATAC-seq data, you can use some built-in data in celloracle. celloracle have a TFinfo made with various kind of tissue/cell-types of mouse ATAC-seq atlas dataset (http://atlas.gs.washington.edu/mouse-atac/).

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

[8]:
```
# 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()
```

[8]:

	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	
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 have a custom class, Oracle. We can use Oracle for the data preprocessing and GRN inference. Oracle object stores all information and do the calculation with its internal functions. We instantiate an Oracle object, then input a gene expression data (anndata) and a TFinfo into the Oracle object.

[9]:
```
# make Oracle object
oracle = co.Oracle()
```

2.1. load gene expression data into oracle object.

When you load scRNA-seq data, please enter the name of clustering data and dimensional reduction data. Clustering data suppose 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.

[18]:
```
# 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']
```

[14]:
```
# The anndata shoud include (1) gene expression count, (2) clustering information,␣
↪(3) trajectory (dimensional reduction embeddings) data.
# Please refer to another notebook for the detail of anndata preprocessing.

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

# Instantiate Oracle object. All calculation will be done in this 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

[15]:
```
# If you can load TF info dataframe with the following code.
oracle.import_TF_data(TF_info_matrix=TFinfo_df)

# Instead of the way avove, you can use TF "dictionary" with the following code.
# 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 arbitrary information about the TF-target gene pair by the manual way.

For example, if there is a study or database about TF-target pair, you can use such information in the following way.

2.3.1. Make TF info dictionary manually

Here, we introduce a way to add TF binding information.

We have a TF binding data in about some TFs. The information is the supplemental table 4 in (http://doi.org/10.1016/j.cell.2015.11.013).

In order to import TF data into Oracle object, we need to convert them into a python dictionary, in which dictionary keys are target gene, and values are regulatory candidate TFs.

[16]:
```
# 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
```

[16]:

	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...

[17]:
```
# Make dictionary: Key is TF, Value is list of Target gene
TF_to_TG_dictionary = {}
```

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```
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 inverse the dictionary above using a utility function in celloracle.
TG_to_TF_dictionary = co.utility.inverse_dictionary(TF_to_TG_dictionary)
```

```
HBox(children=(IntProgress(value=0, max=178), HTML(value='')))
```

2.3.2. Add TF informatio dictionary into the oracle object

[18]:
```
# Add TF information
oracle.addTFinfo_dictionary(TG_to_TF_dictionary)
```

3. Knn imputation

Celloracle uses almost the same strategy as velocyto for visualization of cell transition. This process requires knn imputation.

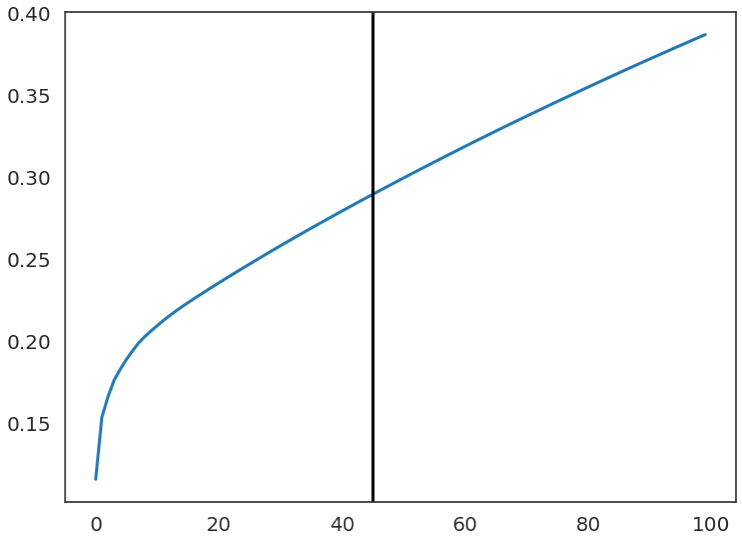
For the Knn imputation, we need PCA and PC selection first.

3.1. PCA

[67]:
```
# 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 number of nearest neighbor for k-nn imputation.

[20]:
```
n_cell = oracle.adata.shape[0]
print(f"cell number is :{n_cell}")
```

```
cell number is :2671
```

[21]:
```
k = int(0.025*n_cell)
print(f"Auto-selected k is :{k}")
```

```
Auto-selected k is :66
```

[22]:
```
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-class: Links, Oracle, TFinfo. You can save such an object using “to_hdf5”.

Pleasae use “load_hdf5” function to load the file.

[23]:
```
# save oracle object.
oracle.to_hdf5("Paul_15_data.celloracle.oracle")
```

[24]:
```
# load file.
# oracle = co.load_hdf5("Paul_15_data.celloracle.oracle")
```

4. GRN calculation

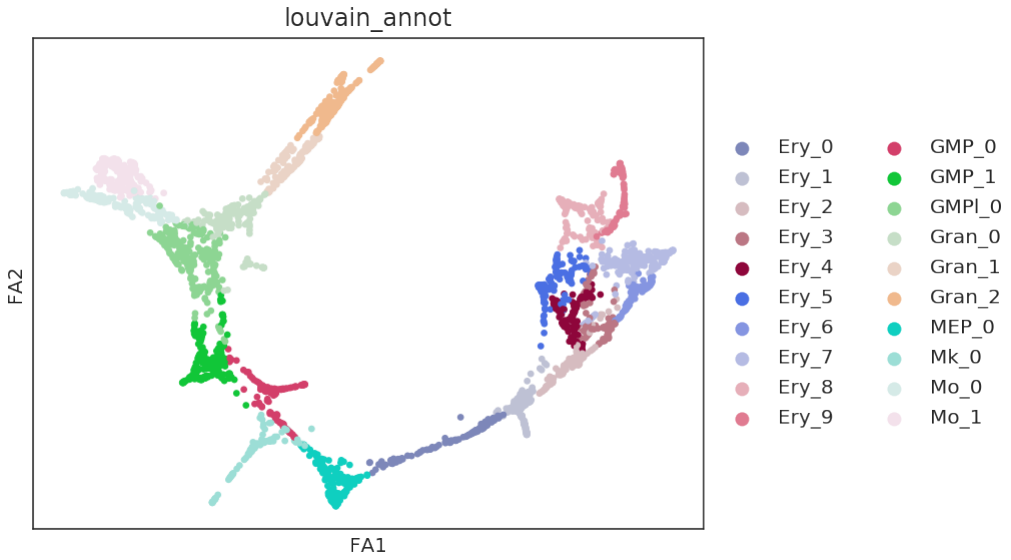
Next step is constructing a cluster-specific GRN for all clusters.

You can calculate GRNs with “get_links” function, and the function returns GRNs as a Links object. Links object stores inferred GRNs and its metadata. You can do network analysis with Links object.

In the example below, we construct GRNs based on “louvain_annot” clustering unit.

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

[43]:
```
# check data
sc.pl.draw_graph(oracle.adata, color="louvain_annot")
```



4.1. Get GRNs

[28]:
```
# 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=2, test_mode=False)
```

```
HBox(children=(IntProgress(value=0, max=20), HTML(value='')))
```

```
inferring GRN for Ery_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_1...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_2...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_3...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_4...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_5...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_6...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_7...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_8...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Ery_9...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for GMP_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for GMP_1...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for GMPl_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Gran_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Gran_1...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Gran_2...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for MEP_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Mk_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Mo_0...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

```
inferring GRN for Mo_1...
method: bagging_ridge
alpha: 10
```

```
HBox(children=(IntProgress(value=0, max=1850), HTML(value='')))
```

4.2. (Optional) Export GRNs

Although celloracle has many functions for network analysis, you can analyze GRNs by yourself. The raw GRN data is stored in the attribute of "links_dict".

For example, you can get GRNs for "Ery_0" cluster with the following commands.

[11]:
```
links.links_dict["Ery_0"]
```

[11]:

	source	target	coef_mean	coef_abs	p	-logp
0	Id2	0610007L01Rik	0.000552	0.000552	5.724488e-01	0.242263

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```
1        Klf2  0610007L01Rik   0.000000  0.000000           NaN  -0.000000
2      Stat5a  0610007L01Rik  -0.005179  0.005179  5.294497e-04   3.276175
3        Elf1  0610007L01Rik   0.002107  0.002107  1.498884e-01   0.824232
4       Gata1  0610007L01Rik  -0.000700  0.000700  5.731063e-01   0.241765
...       ...            ...        ...       ...           ...        ...
74460   Cxxc1            Zyx  -0.004999  0.004999  1.578852e-02   1.801659
74461   Mef2c            Zyx   0.017708  0.017708  3.011616e-07   6.521200
74462    Nfe2            Zyx   0.034433  0.034433  2.548244e-12  11.593759
74463   Nr3c1            Zyx  -0.022663  0.022663  2.265408e-08   7.644854
74464    Ets1            Zyx   0.012826  0.012826  4.813285e-09   8.317558

[74465 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")
```

4.3. (Optional) Change order

Links object have a color information in a attribute, "palette". This information is used for the visualization

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

[16]:
```
# Show the contents of pallete
links.palette
```

[16]:
```
        palette
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
Gran_0  #C6DEC7
Gran_1  #EAD3C6
Gran_2  #F0B98D
MEP_0   #0FCFC0
Mk_0    #9CDED6
Mo_0    #D5EAE7
Mo_1    #F3E1EB
```

[17]:
```
# 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']
```

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```
links.palette = links.palette.loc[order]
links.palette
```

[17]:

	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

5. Network preprocessing

5.1. Filter network edges

Celloracle leverages bagging ridge or Bayesian ridge regression for network inference. These methods provide a network edge strength as a distribution rather than 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 edge based on the p-value.
2. Remove weak network edge. In this tutorial, we pick up 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." So the filtering function keeps raw network information rather than overwriting data. You can come back to the filtering process to filter the data with different parameters if you want.

[32]:
```
links.filter_links(p=0.001, weight="coef_abs", thread_number=2000)
```

5.2. Degree distribution

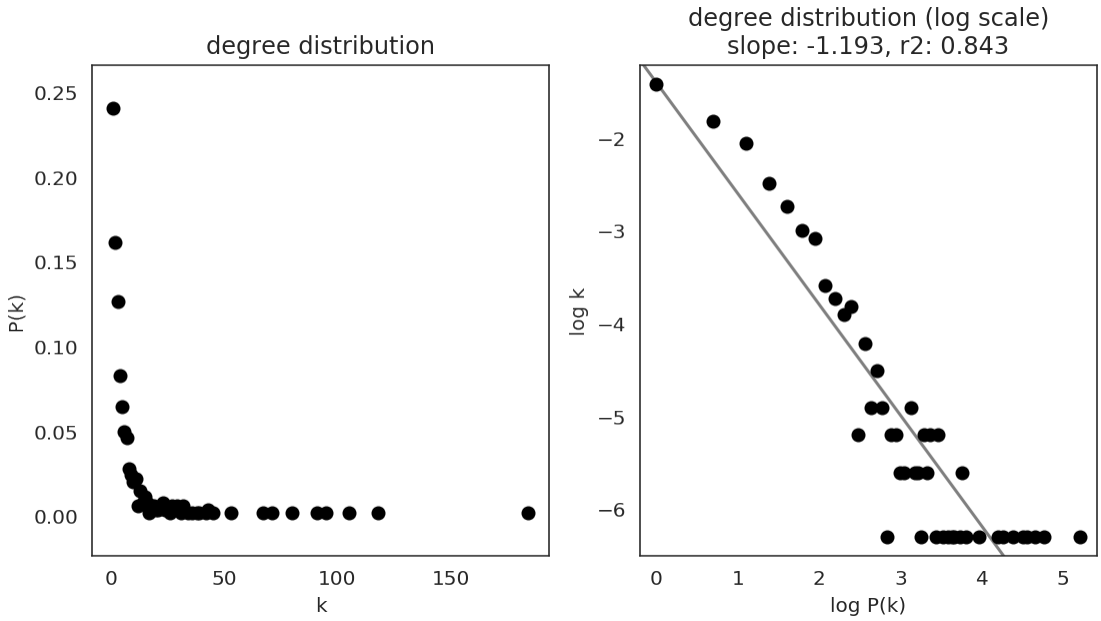
For the first step, we examine network degree distribution. Network degree, which is the number of edges for each node, is one of the important metrics to investigate the network structure (https://en.wikipedia.org/wiki/Degree_distribution).

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

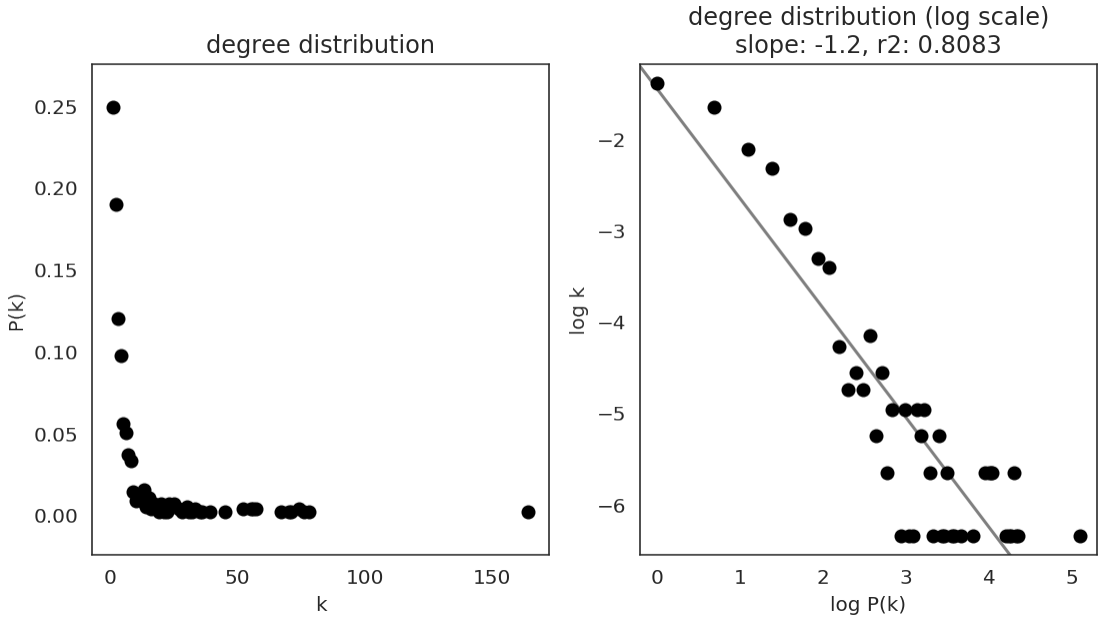
[50]:
```
plt.rcParams["figure.figsize"] = [9, 4.5]
```

[51]:
```
links.plot_degree_distributions(plot_model=True, save=f"{save_folder}/degree_
↪distribution/")
```

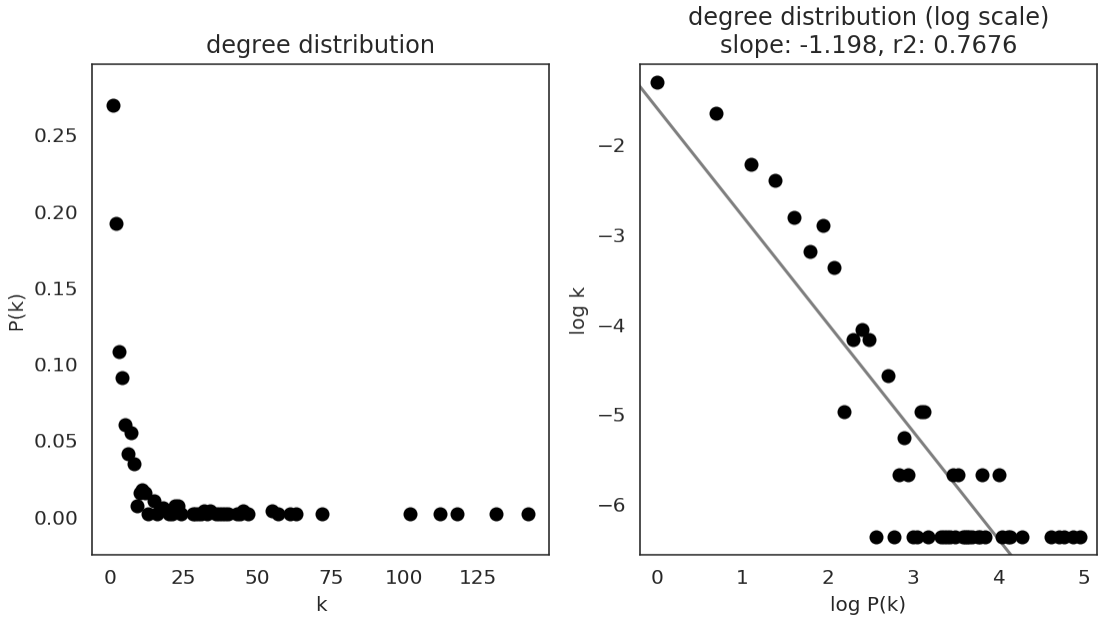
```
Ery_0
```



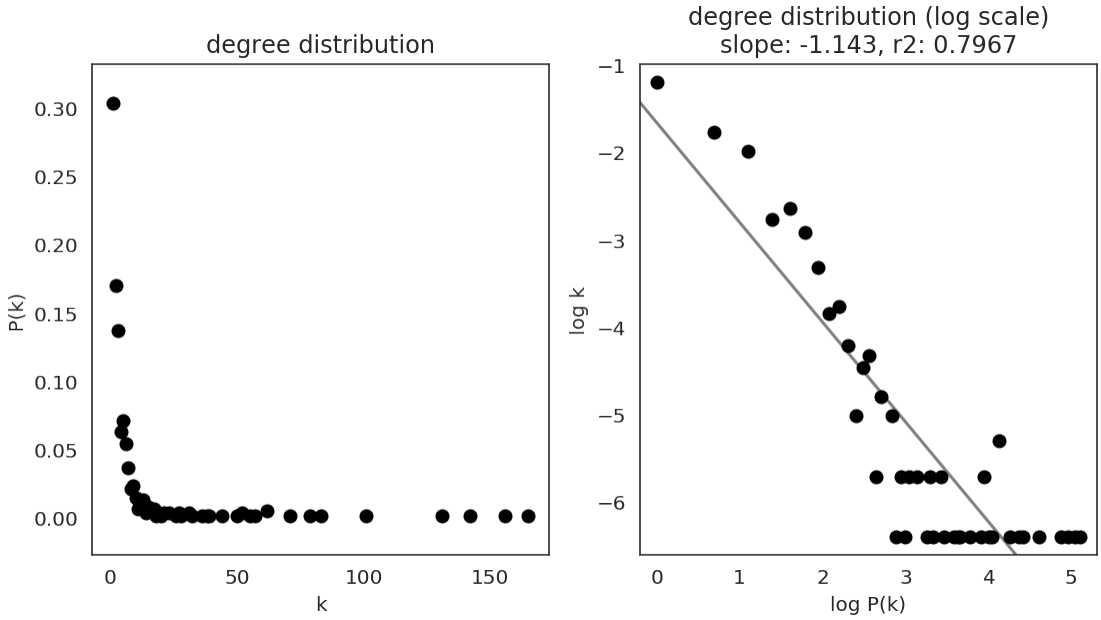

```
Ery_1
```



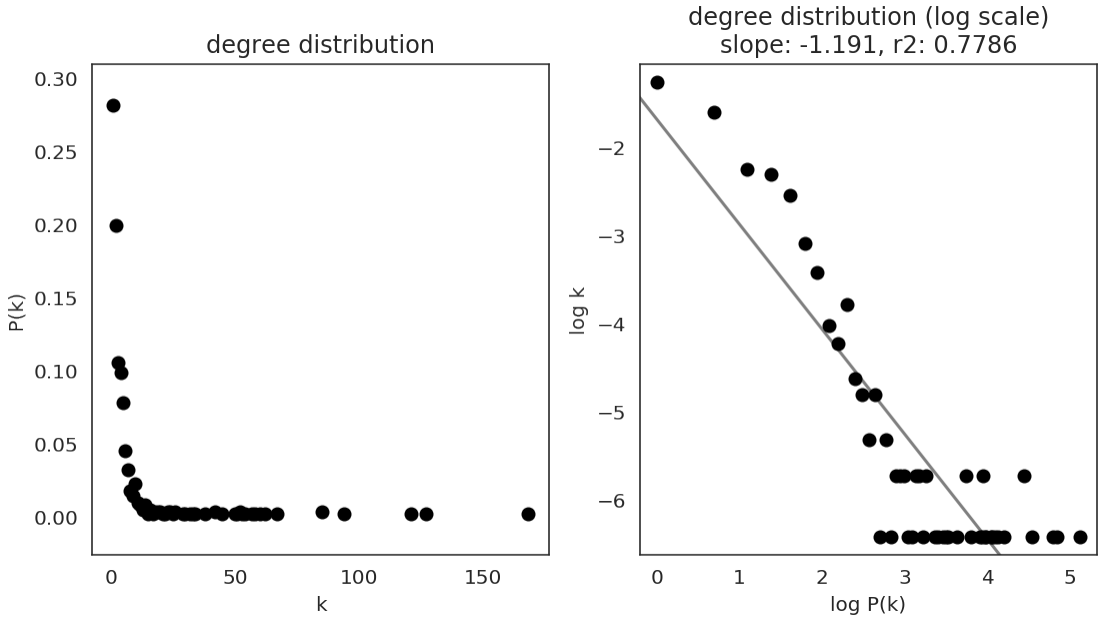

```
Ery_2
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.198, r2: 0.7676
log k
log P(k)

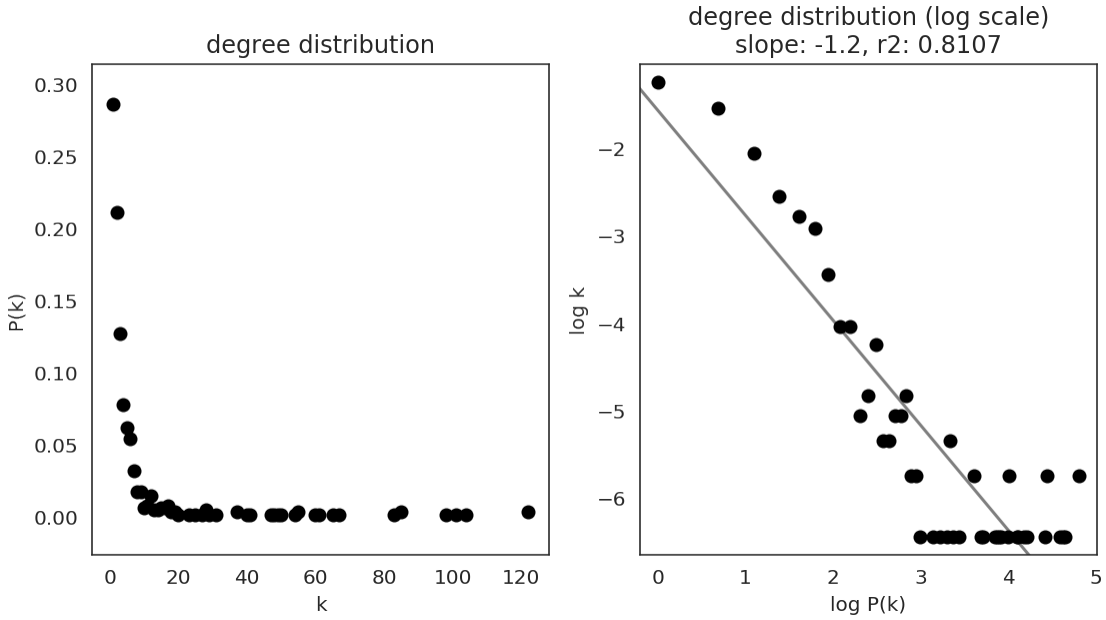
```
Ery_3
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.143, r2: 0.7967
log k
log P(k)

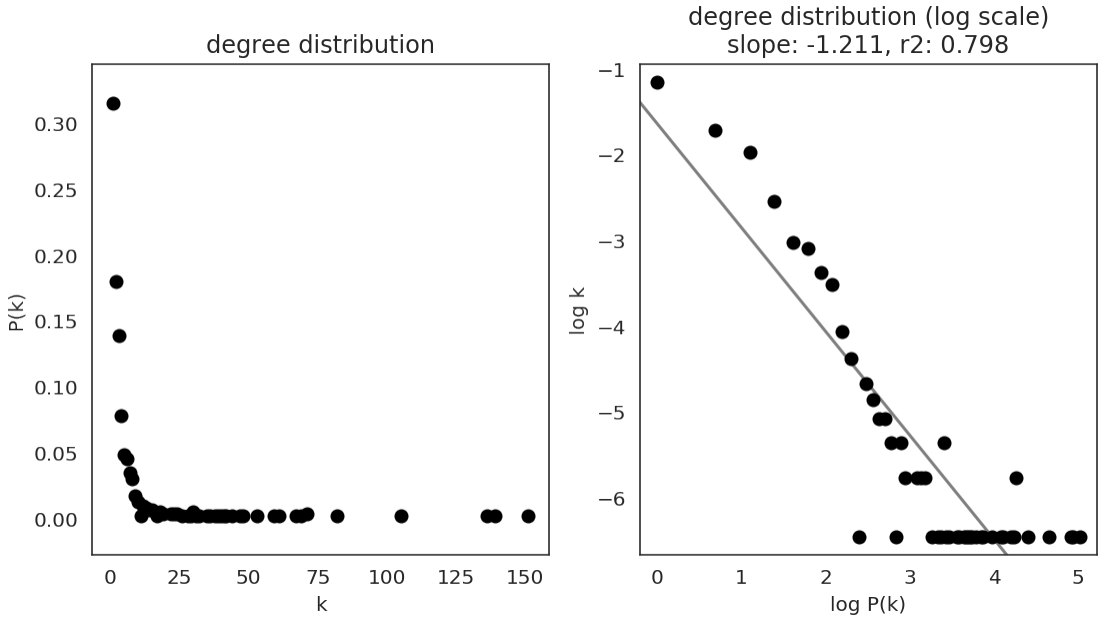
```
Ery_4
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.191, r2: 0.7786
log k
log P(k)

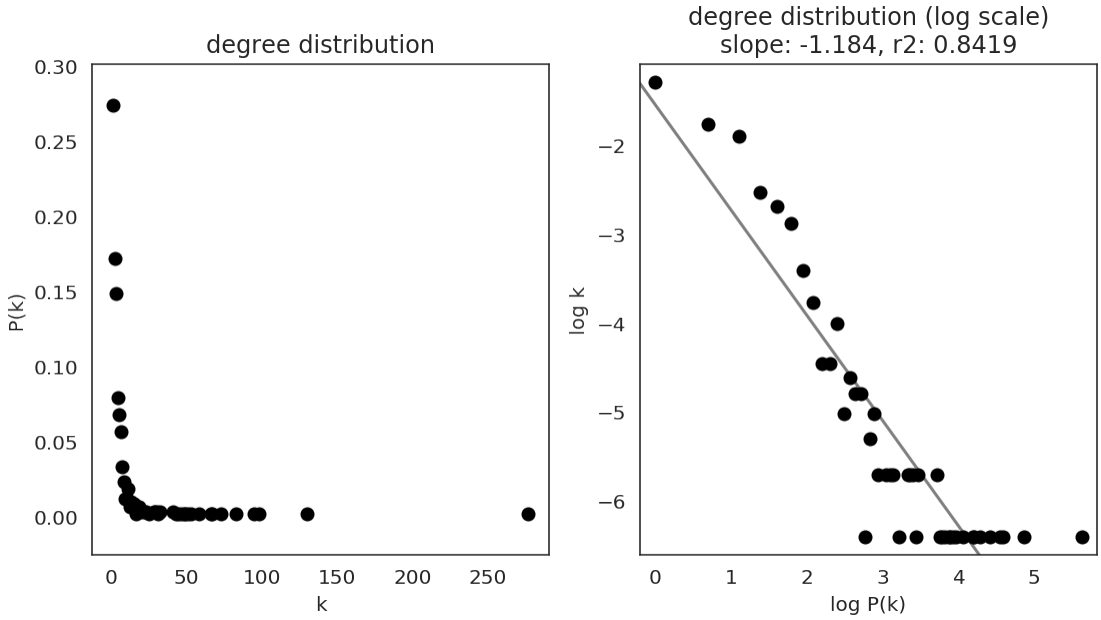
```
Ery_5
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.2, r2: 0.8107
log k
log P(k)

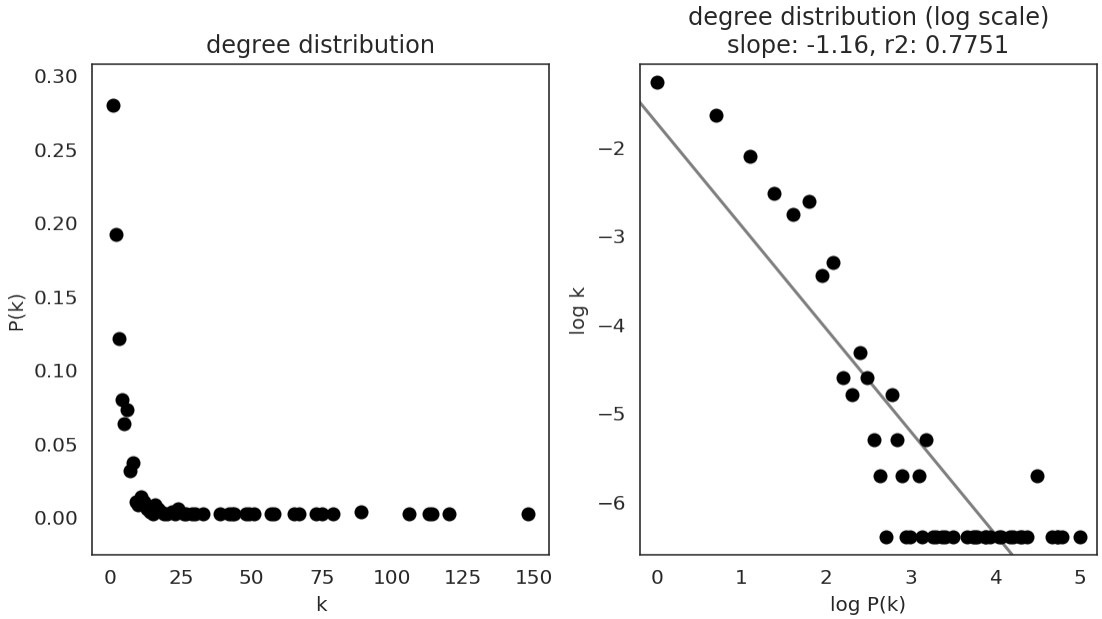
```
Ery_6
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.211, r2: 0.798
log k
log P(k)

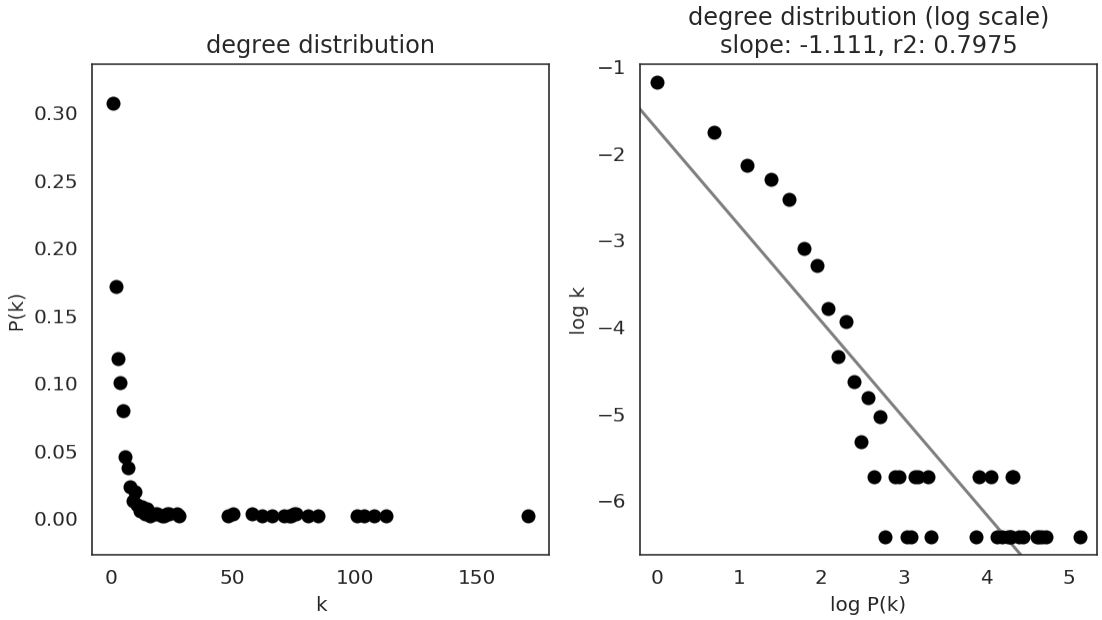
```
Ery_7
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.184, r2: 0.8419
log k
log P(k)

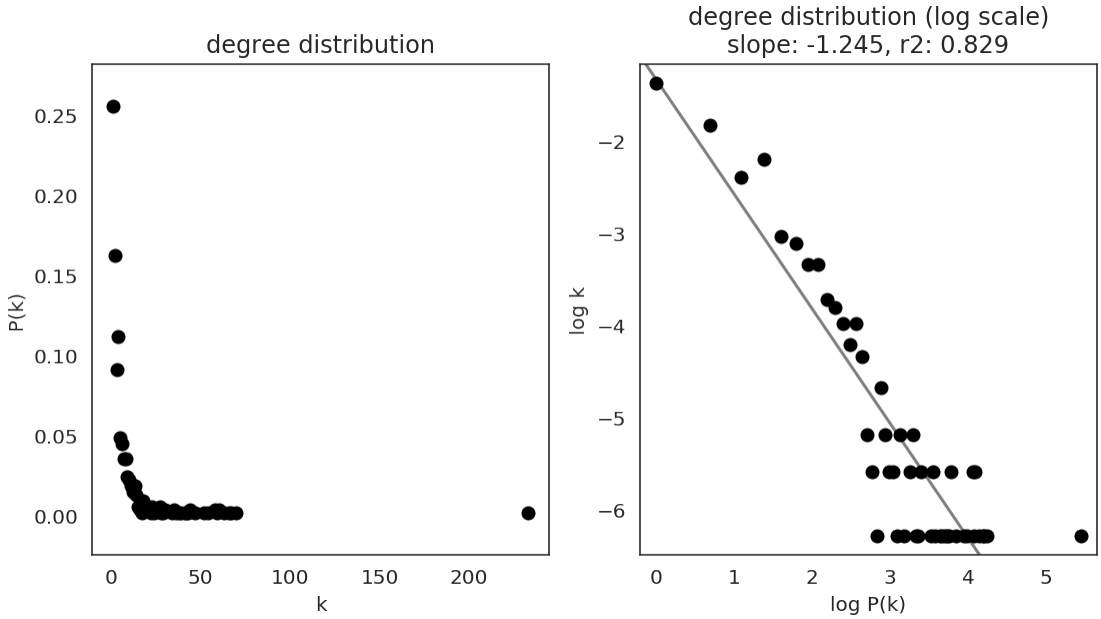
```
Ery_8
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.16, r2: 0.7751
log k
log P(k)

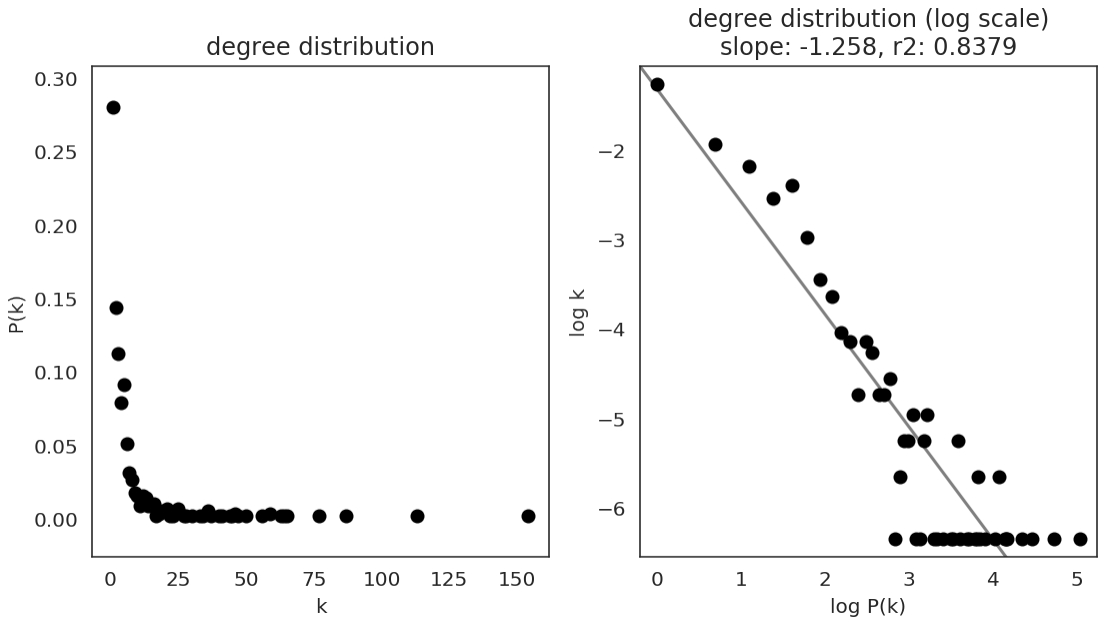
```
Ery_9
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.111, r2: 0.7975
log k
log P(k)

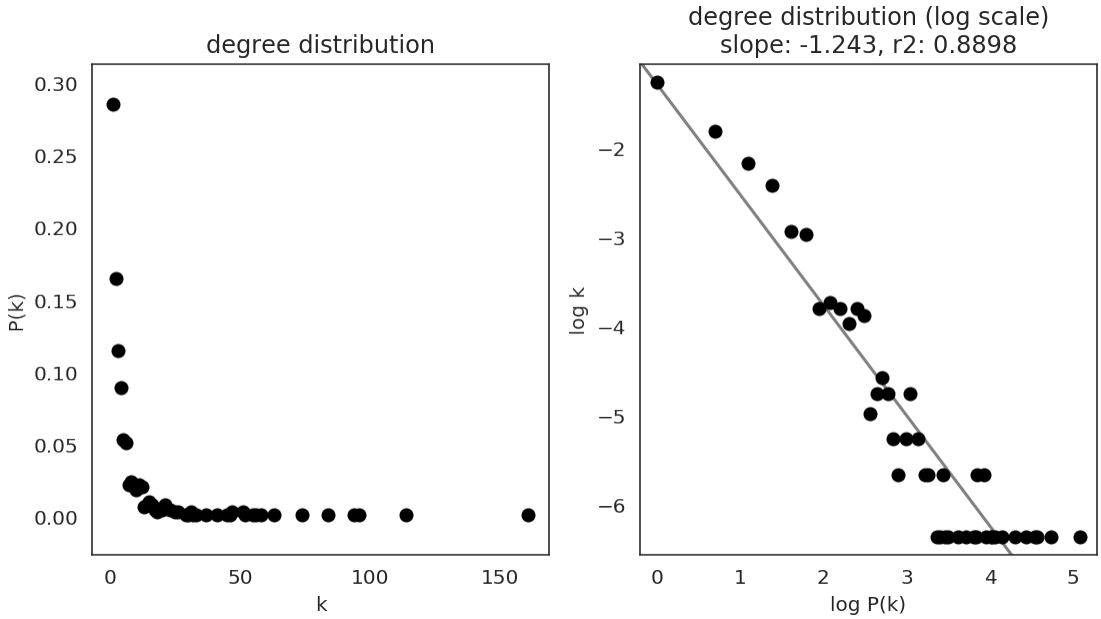
```
GMP_0
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.245, r2: 0.829
log k
log P(k)

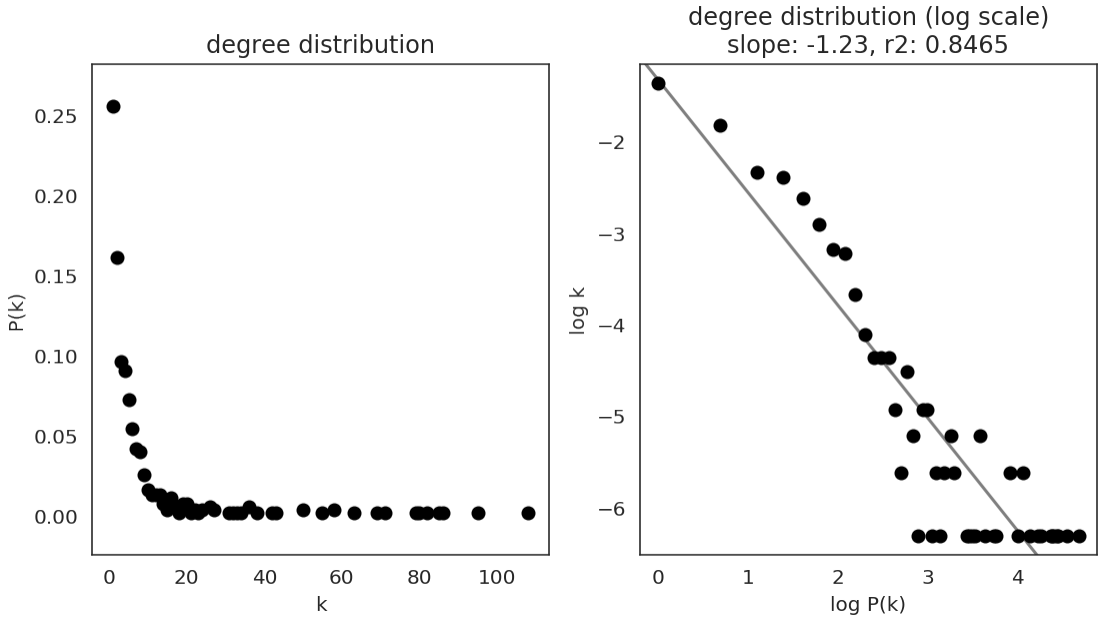
```
GMP_1
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.258, r2: 0.8379
log k
log P(k)

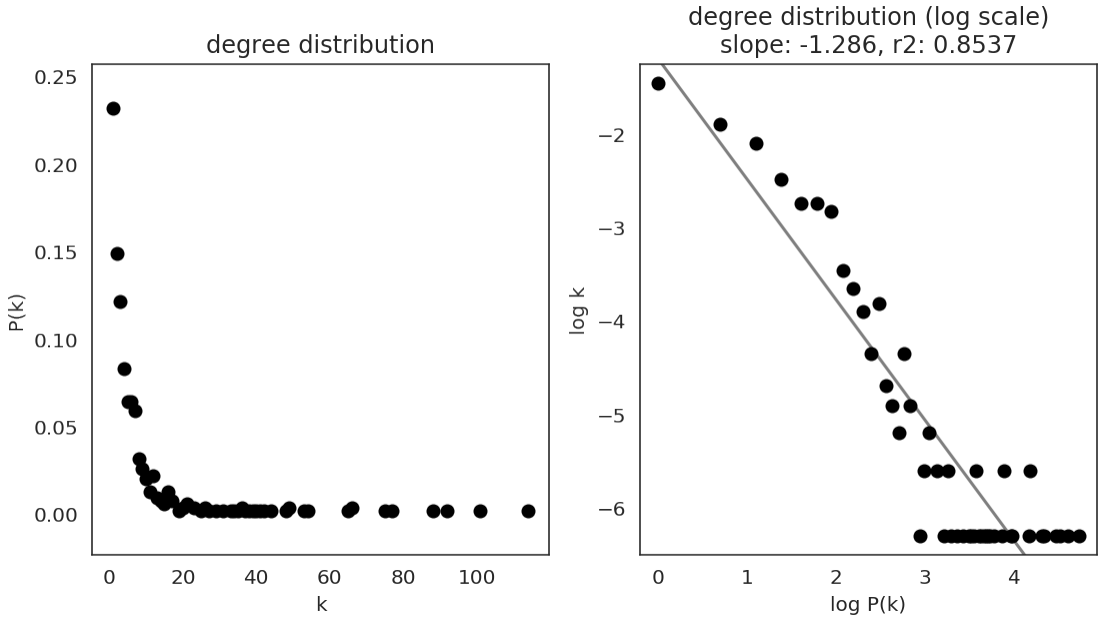
```
GMP1_0
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.243, r2: 0.8898
log k
log P(k)

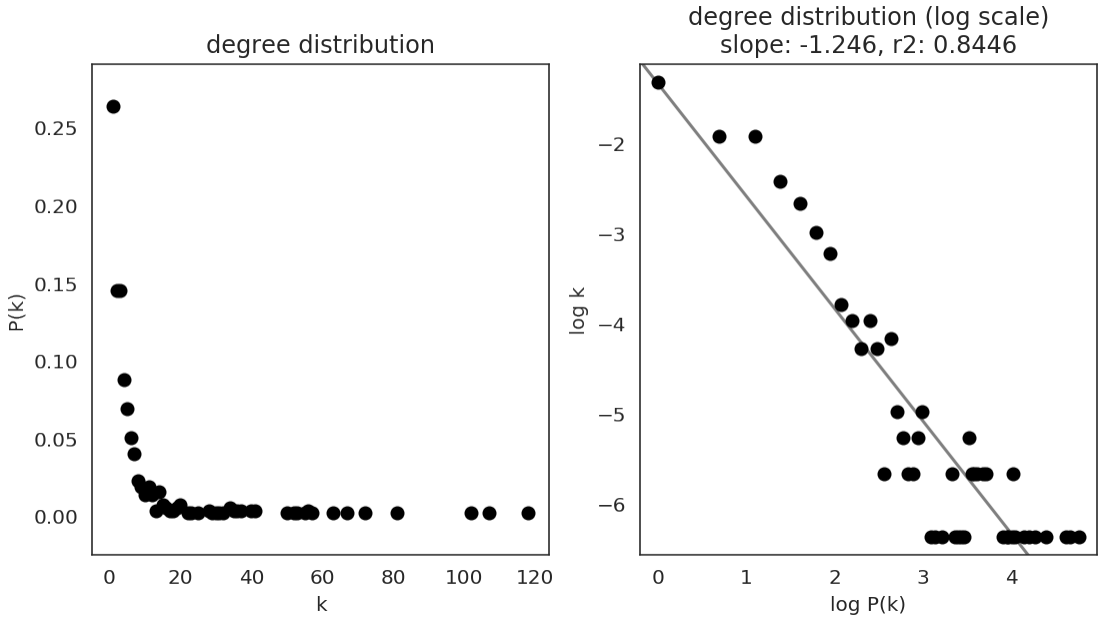
```
Gran_0
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.23, r2: 0.8465
log k
log P(k)

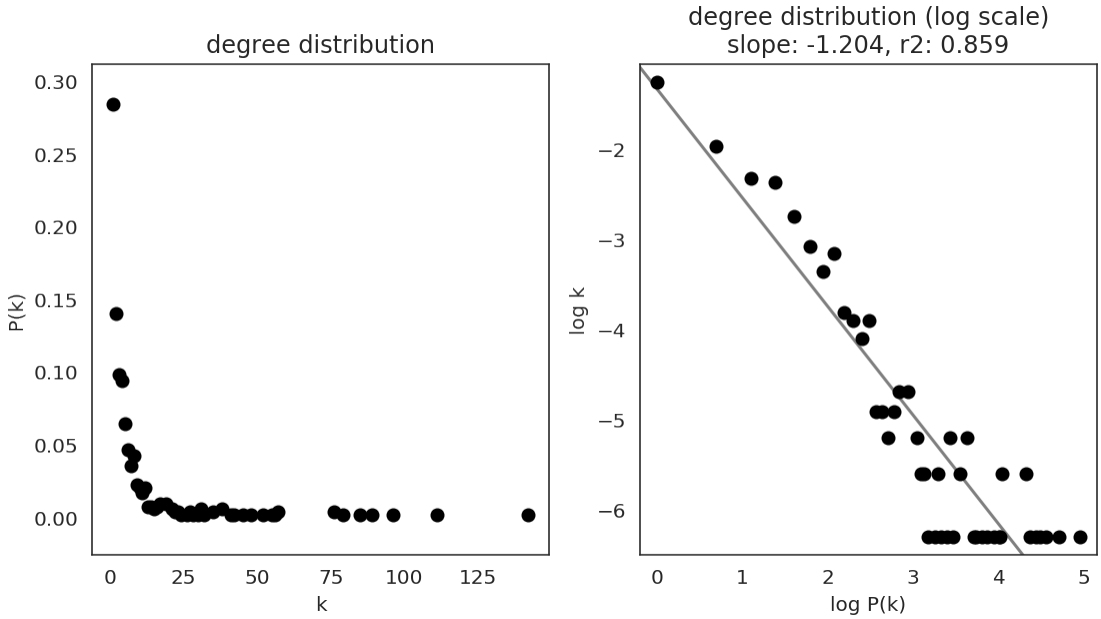
```
Gran_1
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.286, r2: 0.8537
log k
log P(k)

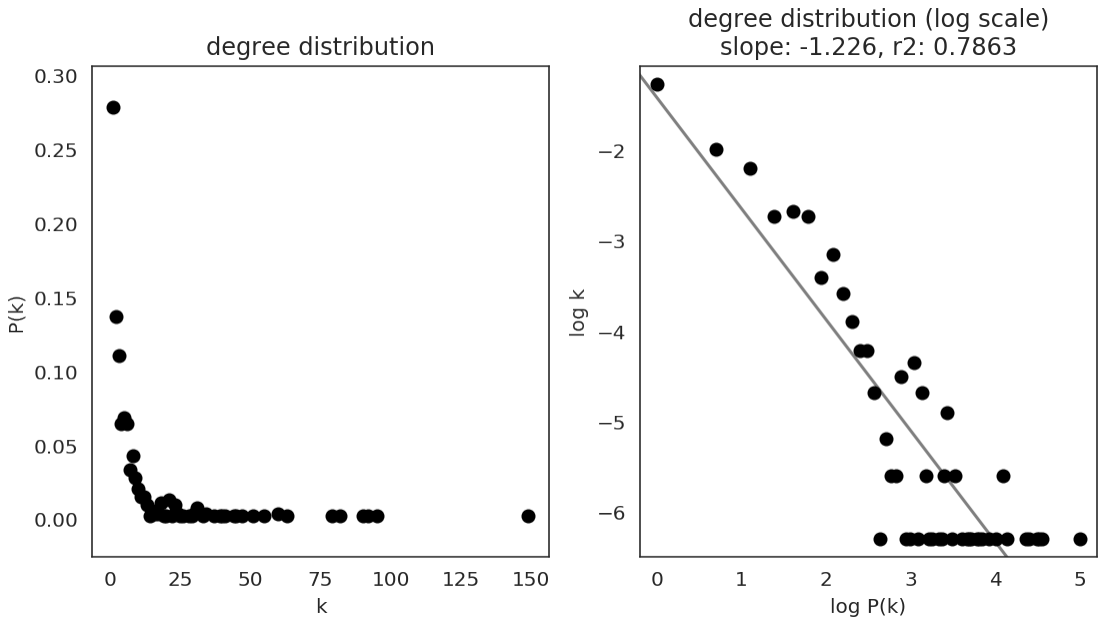
```
Gran_2
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.246, r2: 0.8446
log k
log P(k)

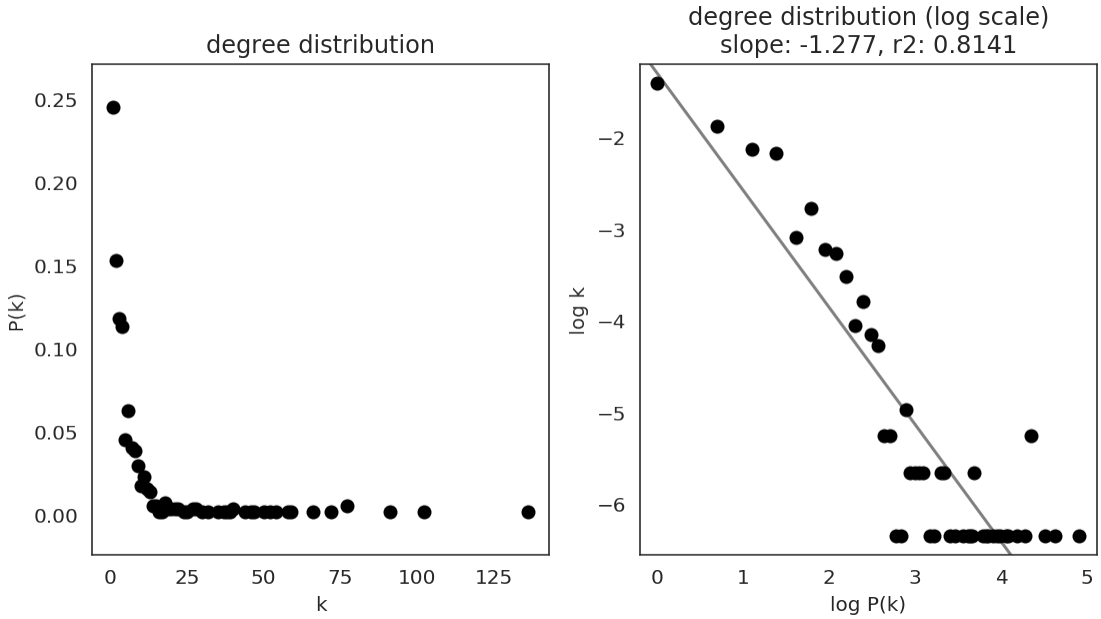
```
MEP_0
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.204, r2: 0.859
log k
log P(k)

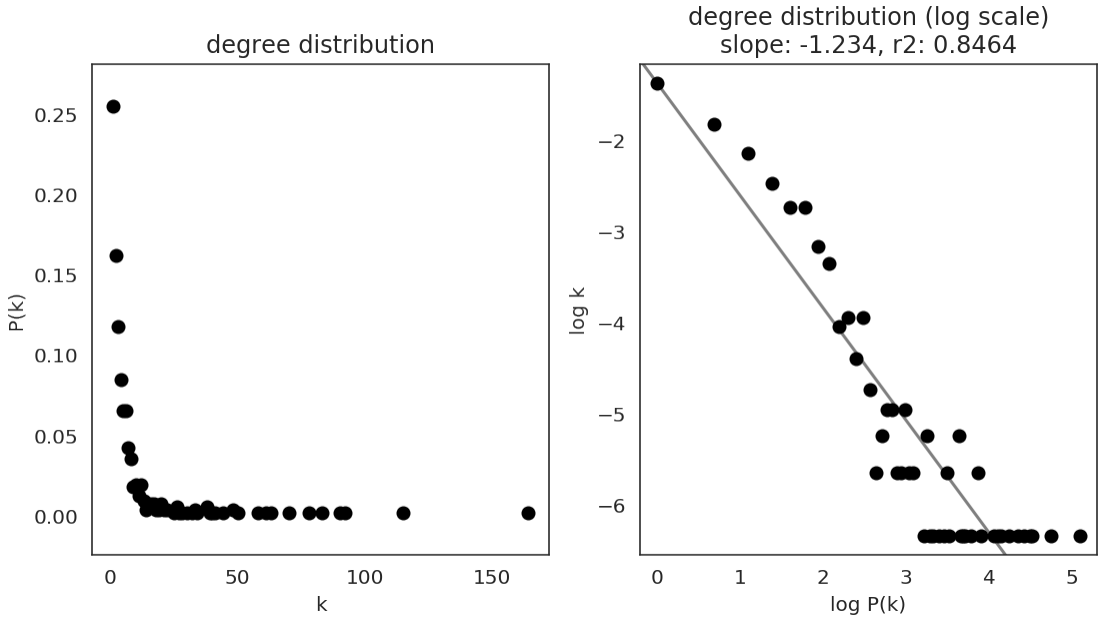
```
Mk_0
```


degree distribution
P(k)
k
degree distribution (log scale)
slope: -1.226, r2: 0.7863
log k
log P(k)

```
Mo_0
```



Mo_1



[52]:
```
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.

[39]:
```
# calculate network scores. It takes several minuts.
links.get_score()
```

```
processing... batch 1/5
Ery_0: finished.
Ery_1: finished.
Ery_2: finished.
Ery_3: finished.
processing... batch 2/5
Ery_4: finished.
Ery_5: finished.
Ery_6: finished.
Ery_7: finished.
processing... batch 3/5
Ery_8: finished.
Ery_9: finished.
GMP_0: finished.
GMP_1: finished.
processing... batch 4/5
GMPl_0: finished.
Gran_0: finished.
Gran_1: finished.
Gran_2: finished.
processing... batch 5/5
MEP_0: finished.
Mk_0: finished.
Mo_0: finished.
Mo_1: finished.
the scores are saved in ./louvain_annot/
```

The score is stored as a attribute "merged_score", and score will also be saved in a folder in your computer.

[57]:
```
links.merged_score.head()
```

[57]:
```
       degree_all  degree_in  degree_out  clustering_coefficient  \
Stat3          91          0          91                0.019780
Mycn           28          0          28                0.002646
Zbtb1          27          0          27                0.005698
E2f4          186          3         183                0.009474
Ybx1           71          9          62                0.027364

       clustering_coefficient_weighted  degree_centrality_all  \
Stat3                         0.020122               0.167279
Mycn                          0.001828               0.051471
Zbtb1                         0.008576               0.049632
E2f4                          0.011623               0.341912
Ybx1                          0.027485               0.130515

       degree_centrality_in  degree_centrality_out  betweenness_centrality  \
Stat3              0.000000               0.167279                       0
Mycn               0.000000               0.051471                       0
Zbtb1              0.000000               0.049632                       0
E2f4               0.005515               0.336397                    2788
Ybx1               0.016544               0.113971                    1153

       closeness_centrality  ...  assortative_coefficient  \
Stat3              0.000013  ...                -0.172207
Mycn               0.000004  ...                -0.172207
Zbtb1              0.000011  ...                -0.172207
E2f4               0.000010  ...                -0.172207
Ybx1               0.000004  ...                -0.172207
```

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```
       average_path_length  community_edge_betweenness  community_random_walk  \
Stat3             2.475244                           1                      1
Mycn              2.475244                           2                      1
Zbtb1             2.475244                           3                      1
E2f4              2.475244                           4                      1
Ybx1              2.475244                           5                     11

       community_eigenvector  module  connectivity  participation  \
Stat3                      1       0      4.052023       0.632291
Mycn                       5       2      2.347146       0.625000
Zbtb1                      5       1      2.275431       0.666667
E2f4                       3       0      8.496354       0.637760
Ybx1                       4       3      5.433857       0.564967

                role  cluster
Stat3  Connector Hub    Ery_0
Mycn       Connector    Ery_0
Zbtb1      Connector    Ery_0
E2f4   Connector Hub    Ery_0
Ybx1   Connector Hub    Ery_0

[5 rows x 22 columns]
```

5.4. Save

Save processed GRN. We use this file in the next notebook; "in silico simulation with GRNs".

[42]:
```
# Save Links object.
links.to_hdf5(file_path="links.celloracle.links")
```

[9]:
```
# You can load files with the following command.
links = co.load_hdf5(file_path="links.celloracle.links")
```

If you are not interested in the network score analysis below and just want to do simulation, you can skip them and go to the next notebook.

6. Network analysis; Network score for each gene

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

6.1. Network score in each cluster

We have calculated several network scores for centrality. 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.

[44]:
```
# check cluster name
links.cluster
```

[44]:
```
['Ery_0',
 'Ery_1',
 'Ery_2',
```

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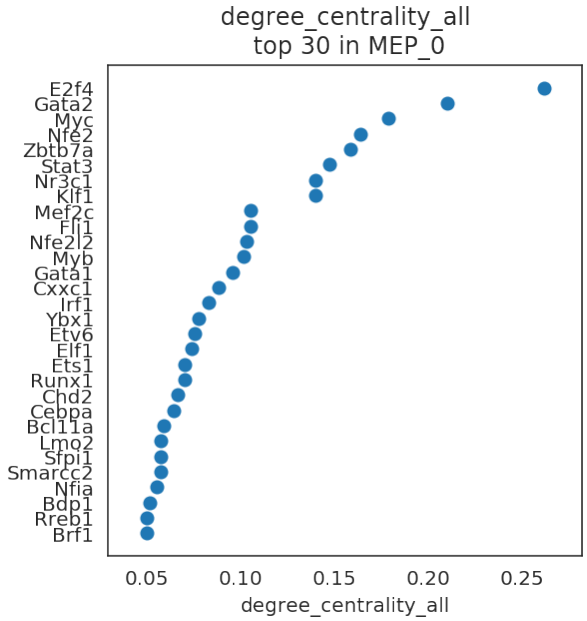
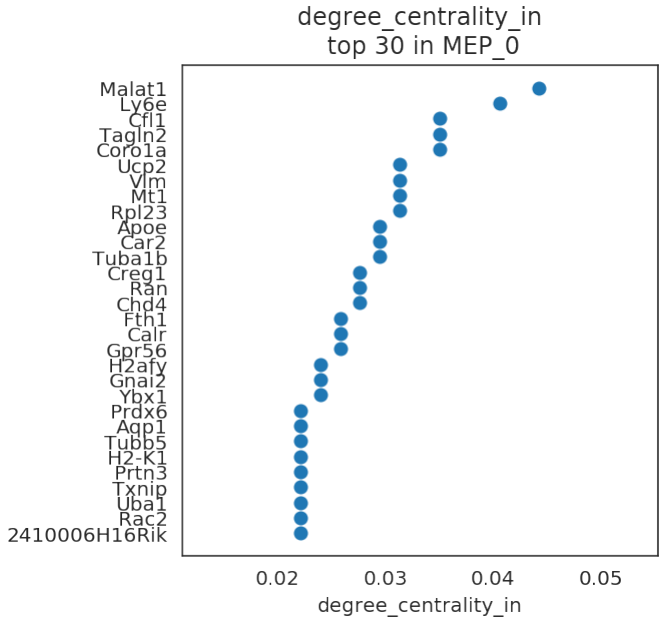
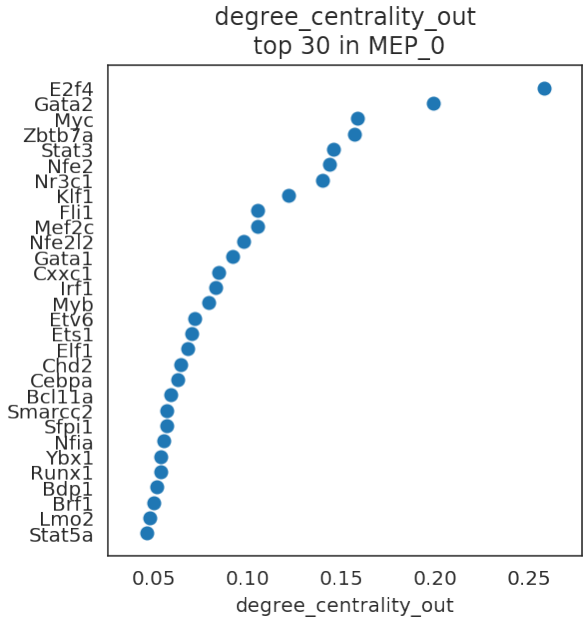
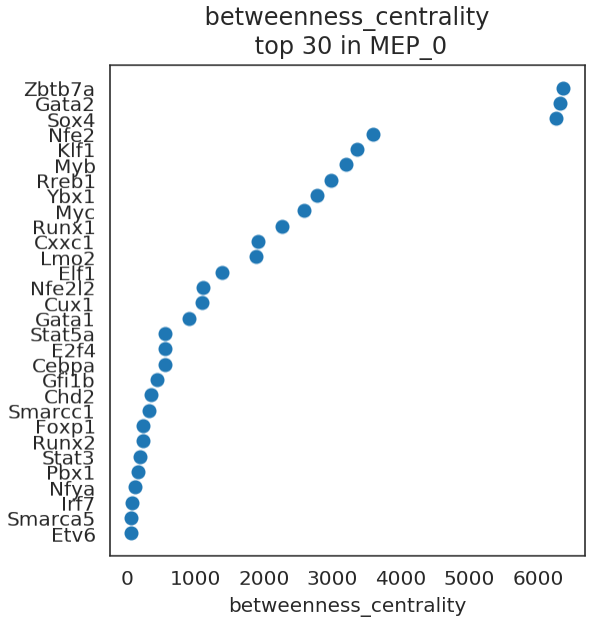
```
 'Ery_3',
 'Ery_4',
 'Ery_5',
 'Ery_6',
 'Ery_7',
 'Ery_8',
 'Ery_9',
 'GMP_0',
 'GMP_1',
 'GMPl_0',
 'Gran_0',
 'Gran_1',
 'Gran_2',
 'MEP_0',
 'Mk_0',
 'Mo_0',
 'Mo_1']
```

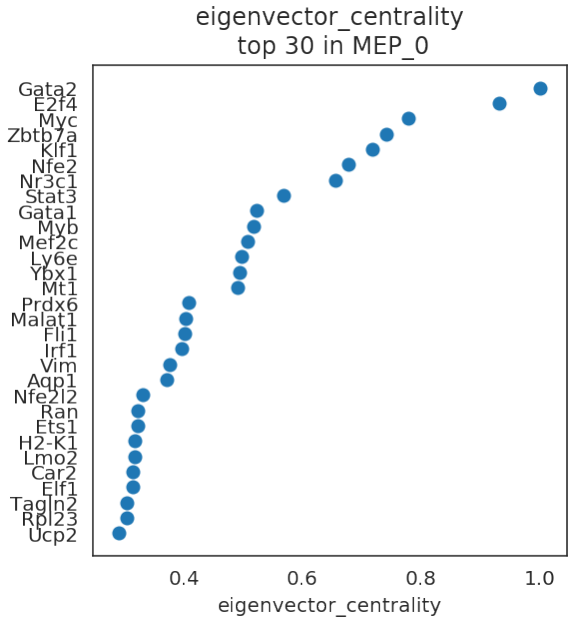
[53]:
```
# Visualize top n-th genes that have high scores.
links.plot_scores_as_rank(cluster="MEP_0", n_gene=30, save=f"{save_folder}/ranked_
↪score")
```

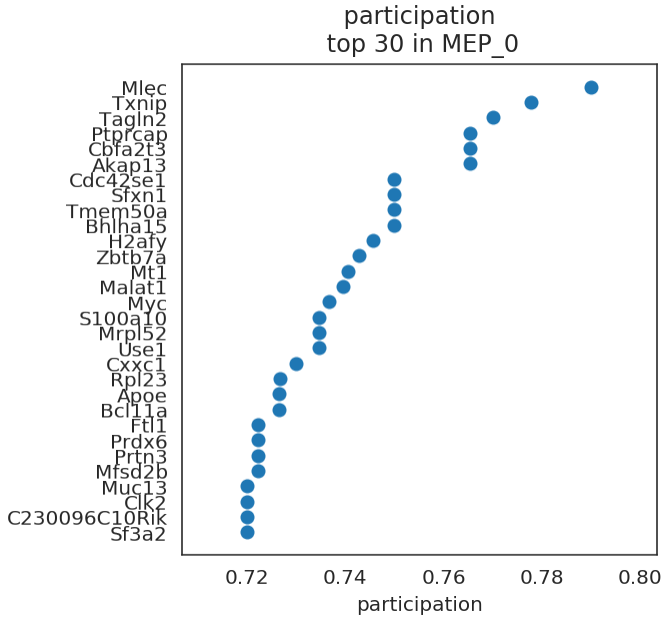



degree_centrality_in
top 30 in MEP_0
Malat1
Ly6e
Cfl1
Tagln2
Coro1a
Ucp2
Vim
Mt1
Rpl23
Apoe
Car2
Tuba1b
Creg1
Ran
Chd4
Fth1
Calr
Gpr56
H2afy
Gnai2
Ybx1
Prdx6
Aqp1
Tubb5
H2-K1
Prtn3
Txnip
Uba1
Rac2
2410006H16Rik
0.02
0.03
0.04
0.05
degree_centrality_in


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
Spi1
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

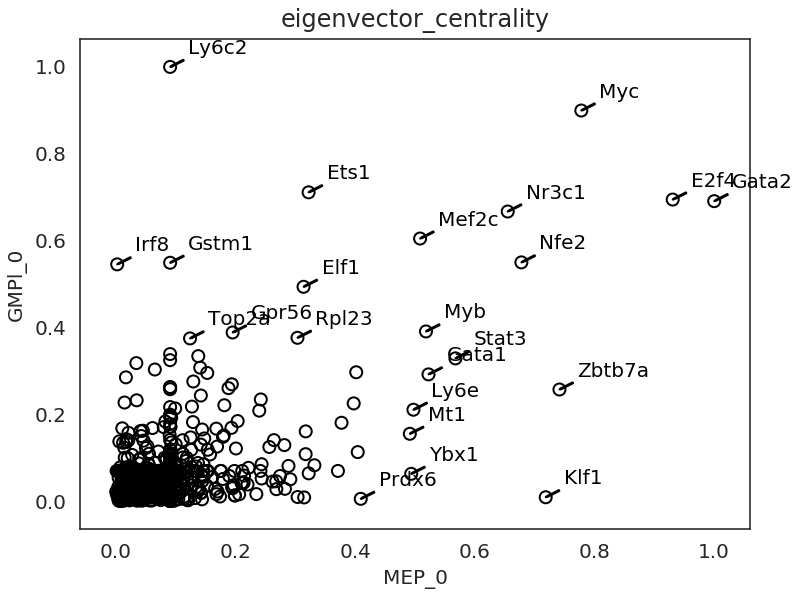

eigenvector_centrality
top 30 in MEP_0
Gata2
E2f4
Myc
Zbtb7a
Klf1
Nfe2
Nr3c1
Stat3
Gata1
Myb
Mef2c
Ly6e
Ybx1
Mt1
Prdx6
Malat1
Fli1
Irf1
Vim
Aqp1
Nfe2l2
Ran
Ets1
H2-K1
Lmo2
Car2
Elf1
Tagln2
Rpl23
Ucp2
0.4
0.6
0.8
1.0
eigenvector_centrality



6.2. Network score comparison between two clusters

By comparing network scores between two clusters, we can analyze the difference of 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))
links.plot_score_comparison_2D(value="betweenness_centrality",
```

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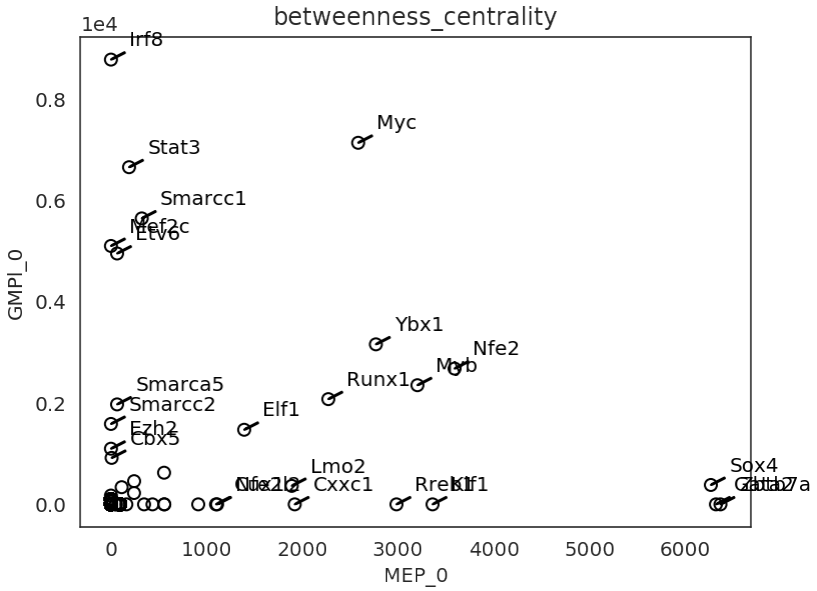
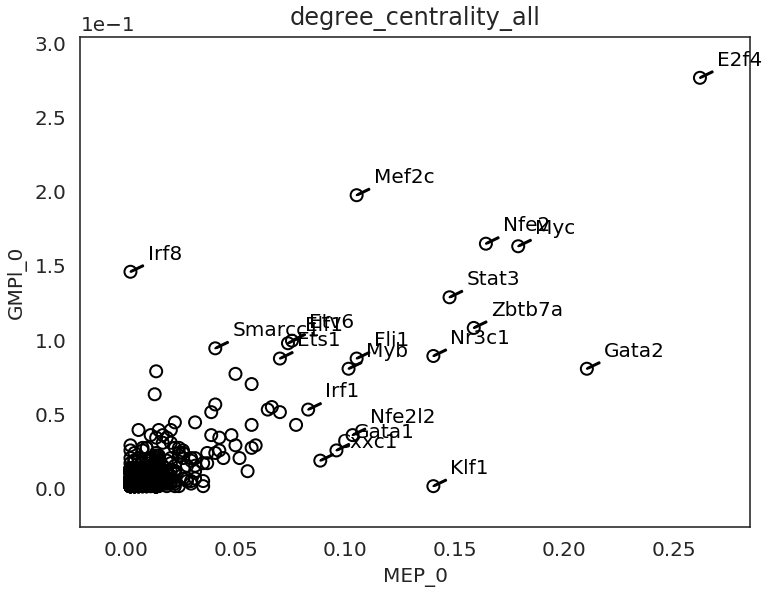
```
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f"{save_folder}/score_comparison")
```



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



6.3. Network score dynamics

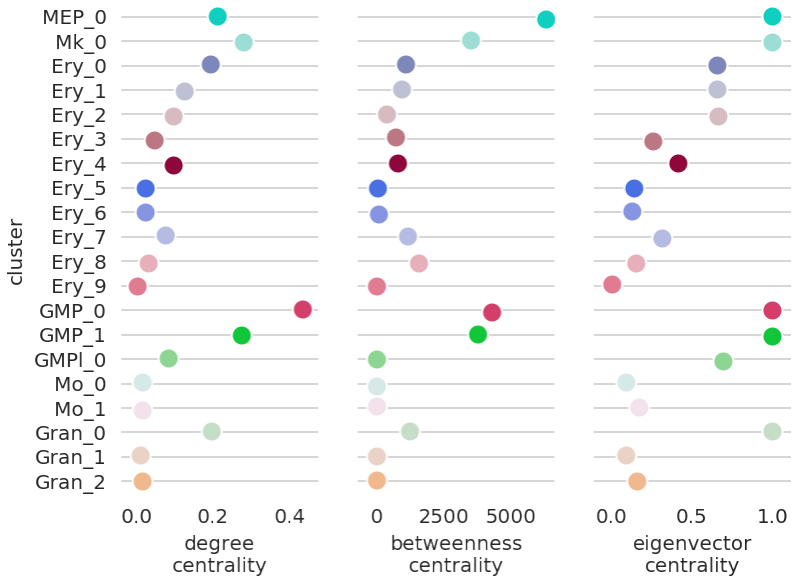
In the following session, we focus on how network score for gene changes during the differentiation.

We make some graph to show an example of how to use functions using Gata2 gene.

Gata2 is known to play an essential role in the early progenitor population; MEP, GMP. The following results can recapitulate the Gata2 feature.

[57]:
```
# Visualize Gata2 network score dynamics
links.plot_score_per_cluster(goi="Gata2", save=f"{save_folder}/network_score_per_gene/
↪")
```

```
Gata2
```



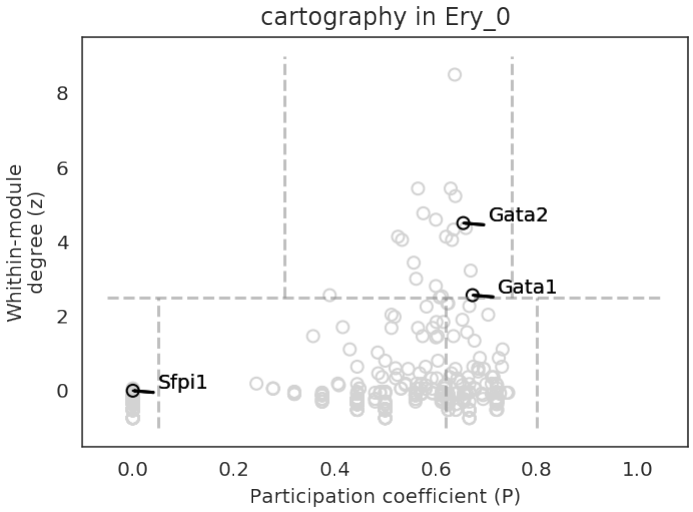
6.4. Gene cartography analysis

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 per each gene. Please read the paper for the details of gene cartography (https://www.nature.com/articles/nature03288)

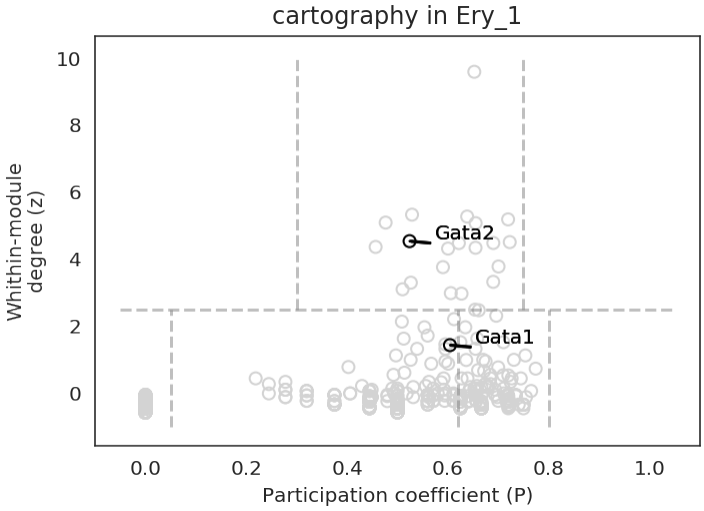
The gene cartography will be calculated for the GRN in each cluster. Thus we can know how it change by comparing the the score between clusters.

[58]:
```
# plot cartography as a scatter plot
links.plot_cartography_scatter_per_cluster(scatter=True,
                                           kde=False,
                                           gois=["Gata1", "Gata2", "Sfpi1"],
                                           auto_gene_annot=False,
                                           args_dot={"n_levels": 105},
                                           args_line={"c":"gray"}, save=f"{save_
↪folder}/cartography")
```

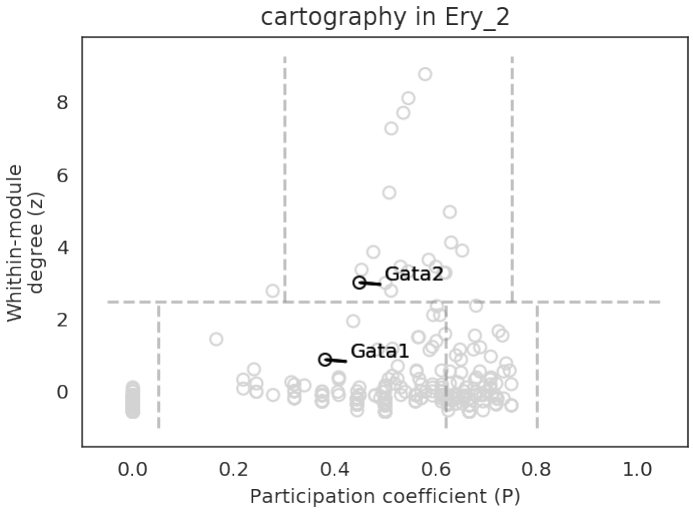
```
Ery_0
```


cartography in Ery_0
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

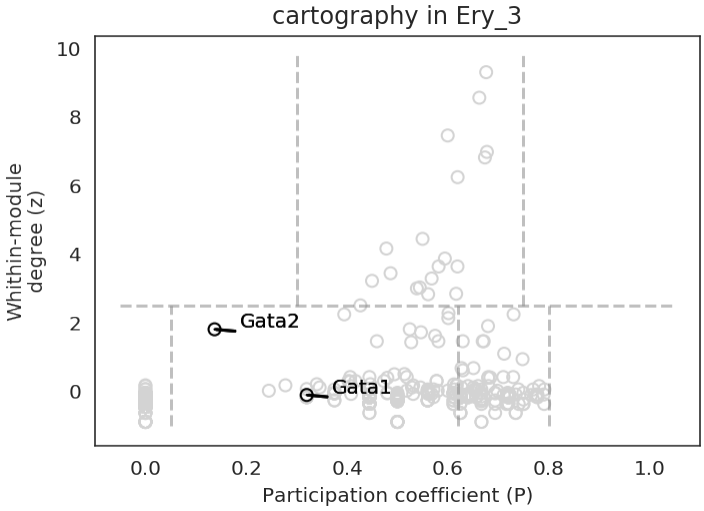
```
Ery_1
```


cartography in Ery_1
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Gata1
0
2
4
6
8
10
0.0
0.2
0.4
0.6
0.8
1.0

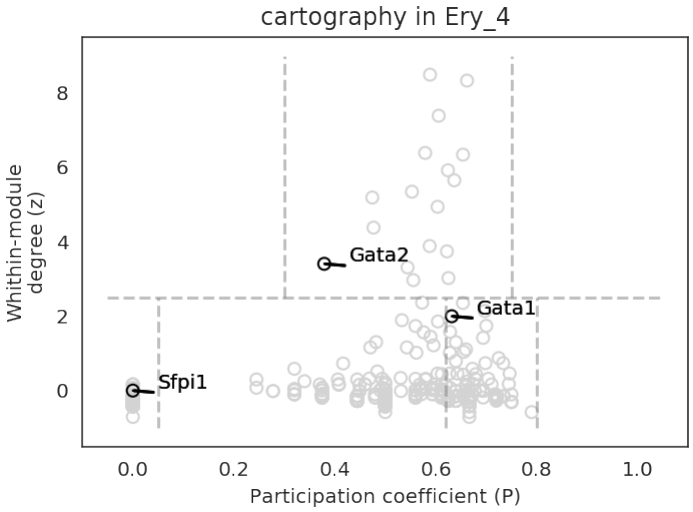
```
Ery_2
```


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

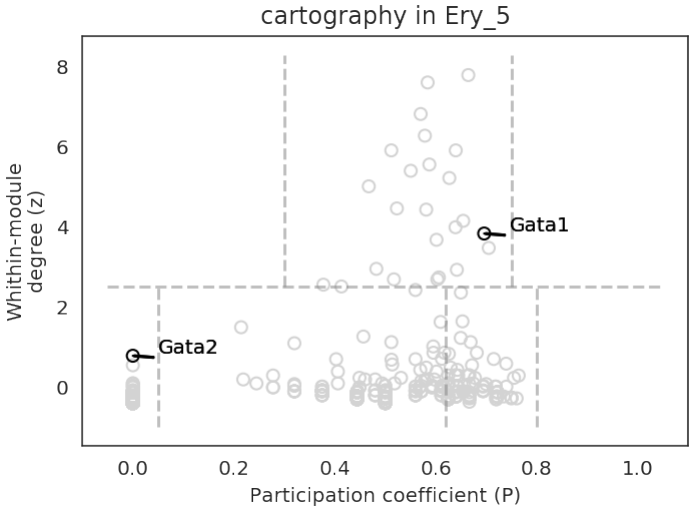
```
Ery_3
```


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

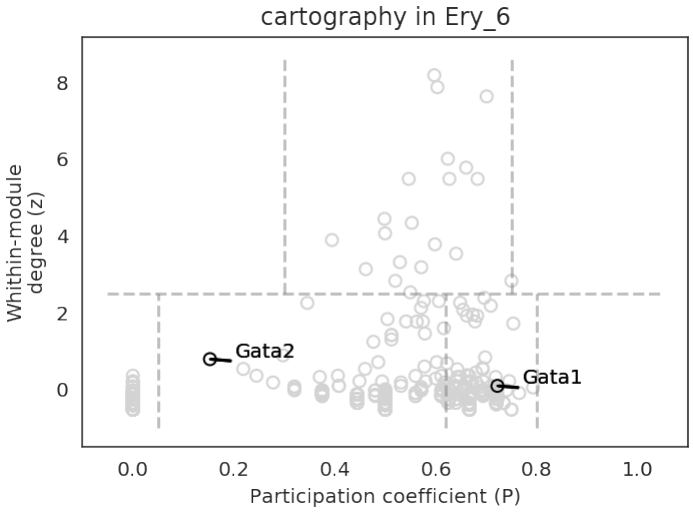
```
Ery_4
```


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

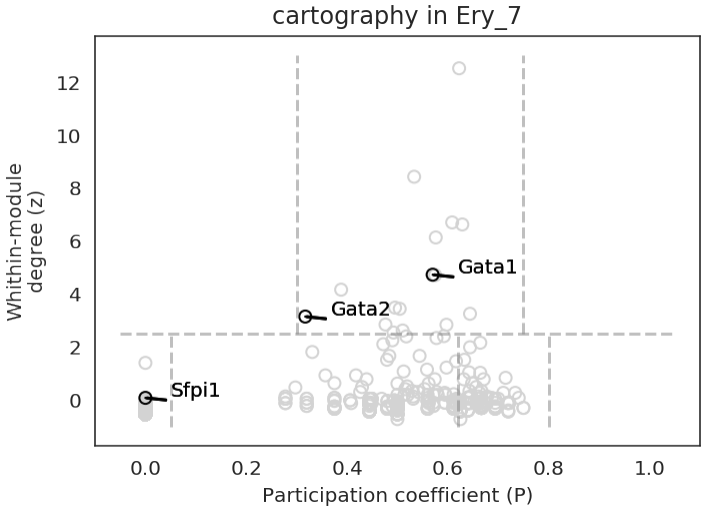
```
Ery_5
```


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

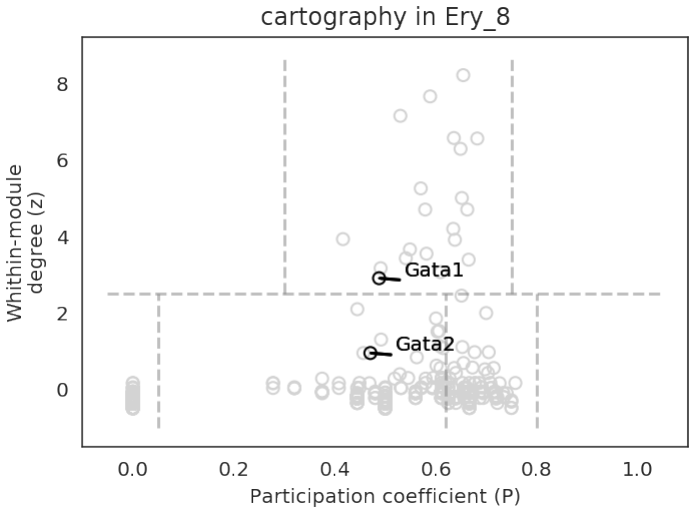
```
Ery_6
```


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

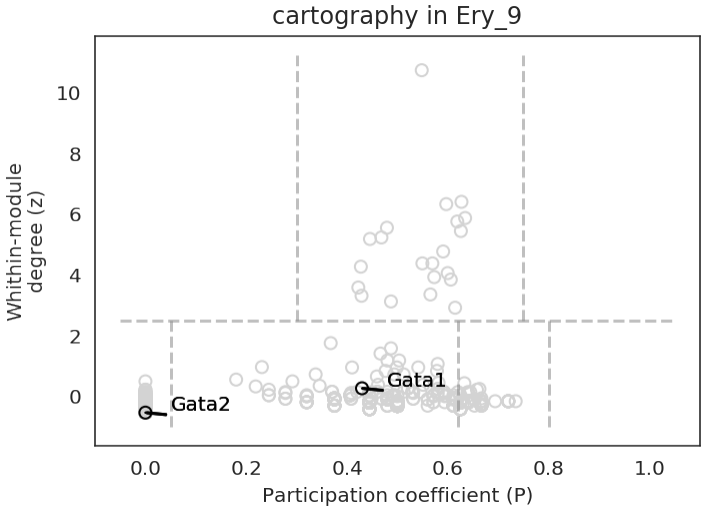
```
Ery_7
```


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

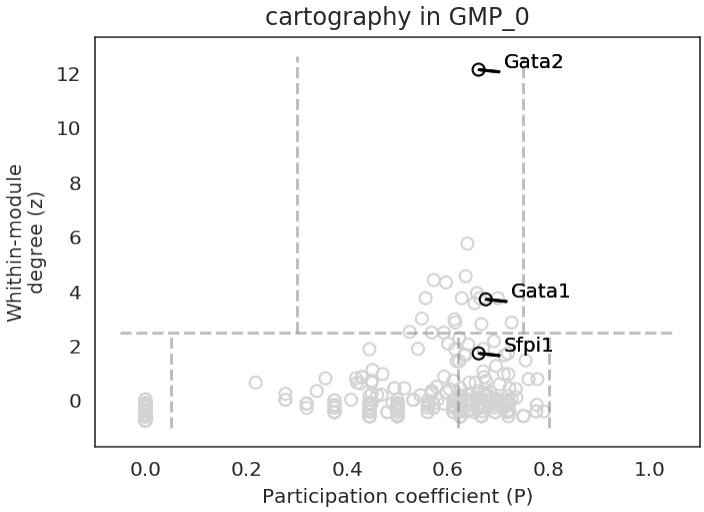
```
Ery_8
```


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

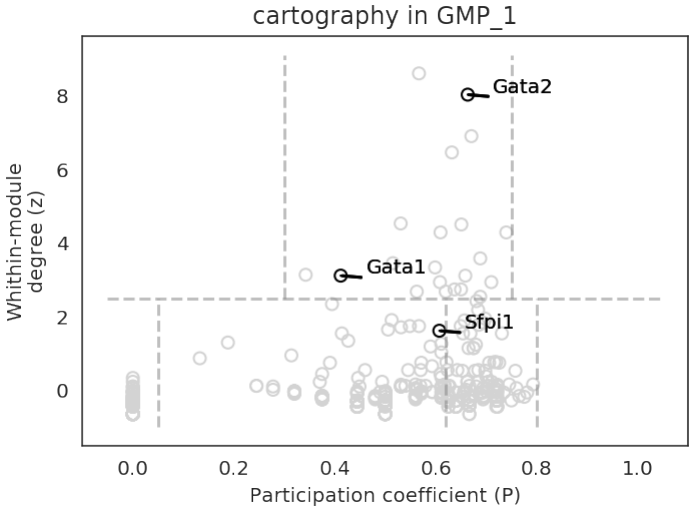
```
Ery_9
```


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

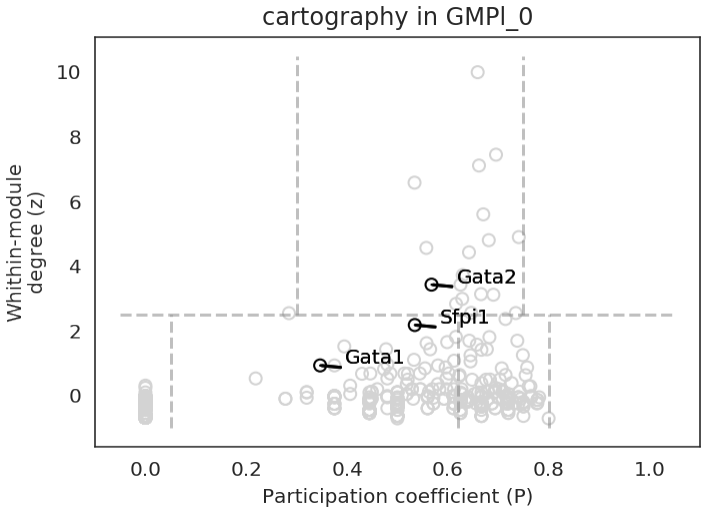
```
GMP_0
```


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

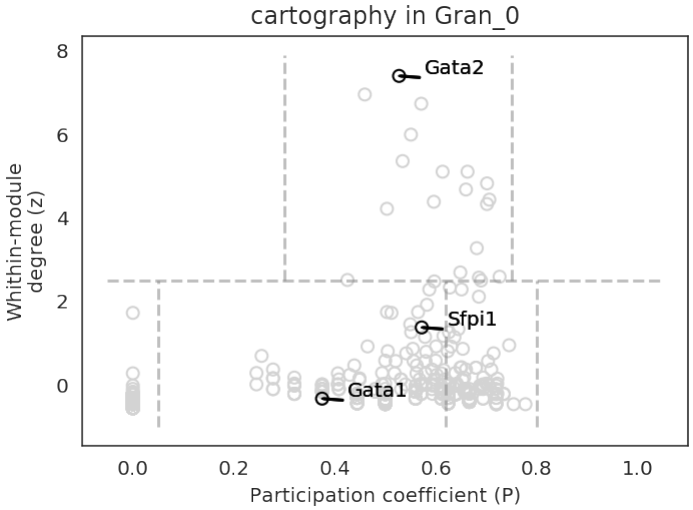
```
GMP_1
```


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

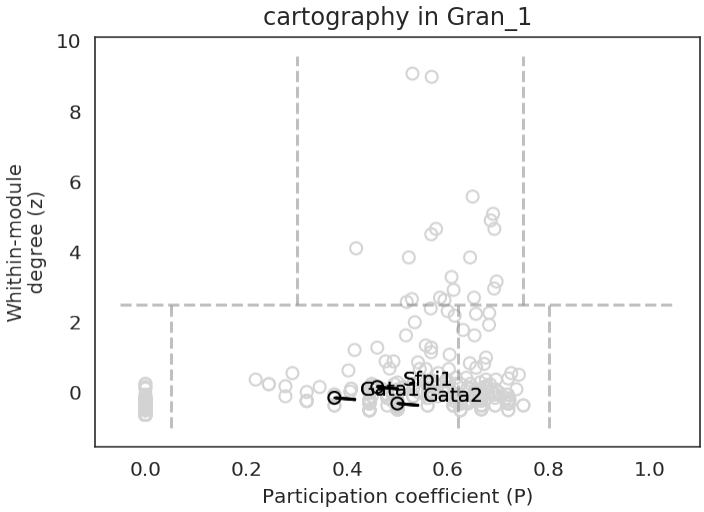
```
GMP1_0
```


cartography in GMPl_0
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Sfpi1
Gata1
0
2
4
6
8
10
0.0
0.2
0.4
0.6
0.8
1.0

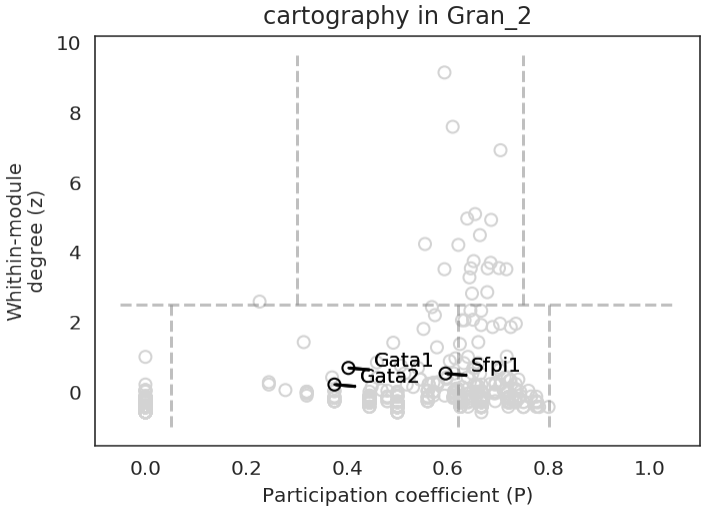
```
Gran_0
```


cartography in Gran_0
Whithin-module degree (z)
Participation coefficient (P)
Gata2
Sfpi1
Gata1
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0

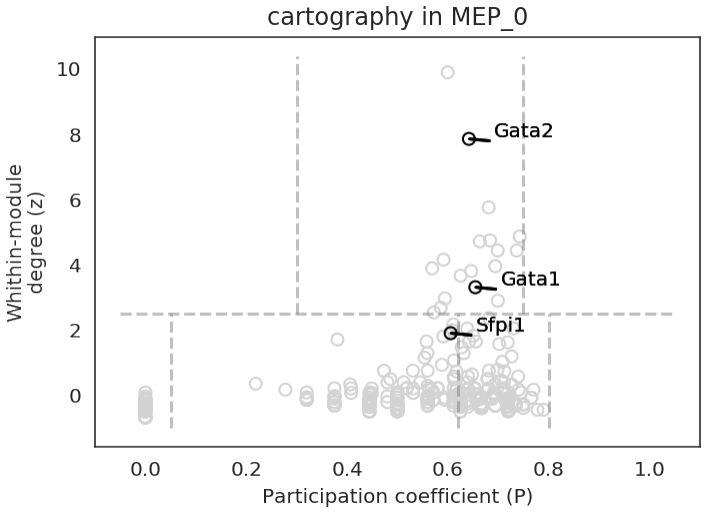
```
Gran_1
```


cartography in Gran_1
Whithin-module degree (z)
Participation coefficient (P)
Sfpi1
Gata1
Gata2

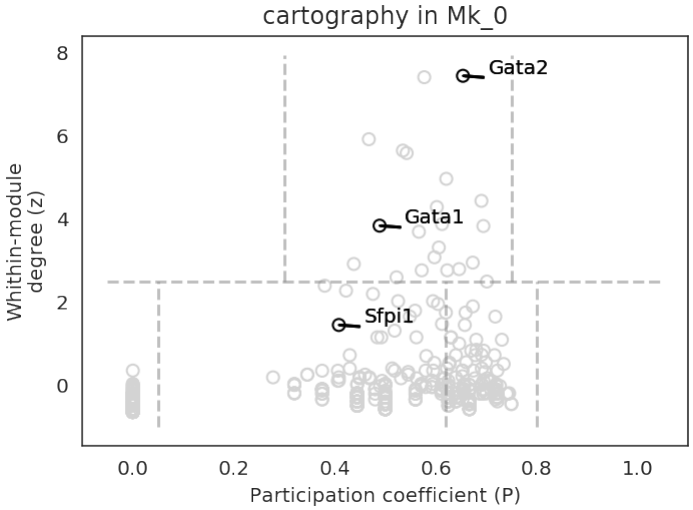
```
Gran_2
```


cartography in Gran_2
Whithin-module degree (z)
Participation coefficient (P)
Gata1
Gata2
Sfpi1

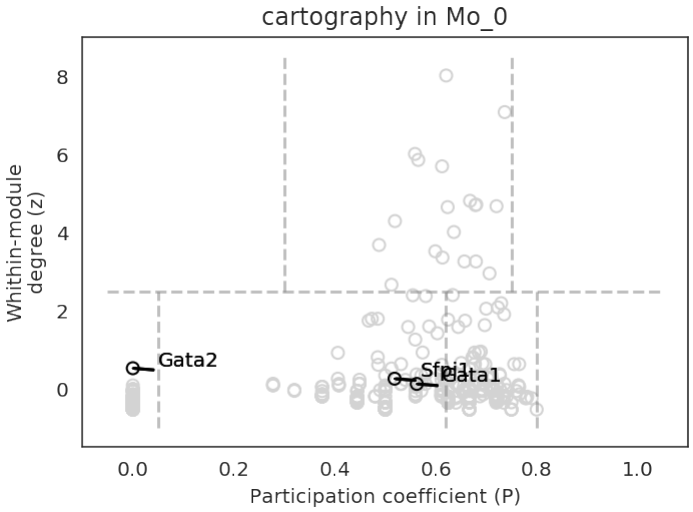
```
MEP_0
```


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

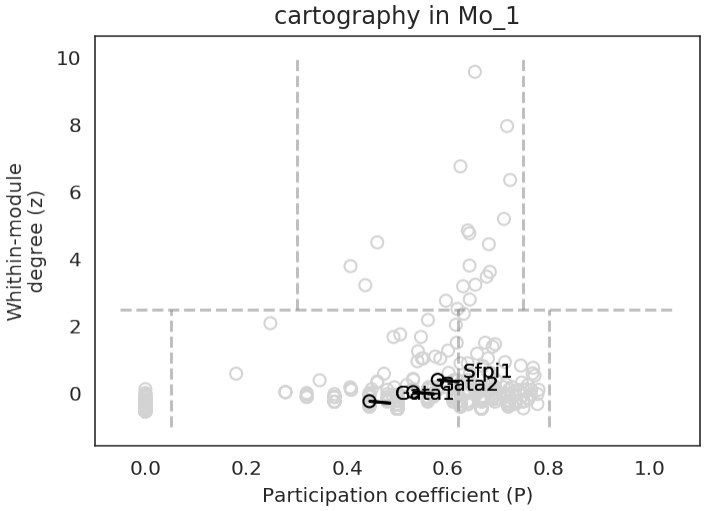
```
Mk_0
```


cartography in Mk_0
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

```
Mo_0
```

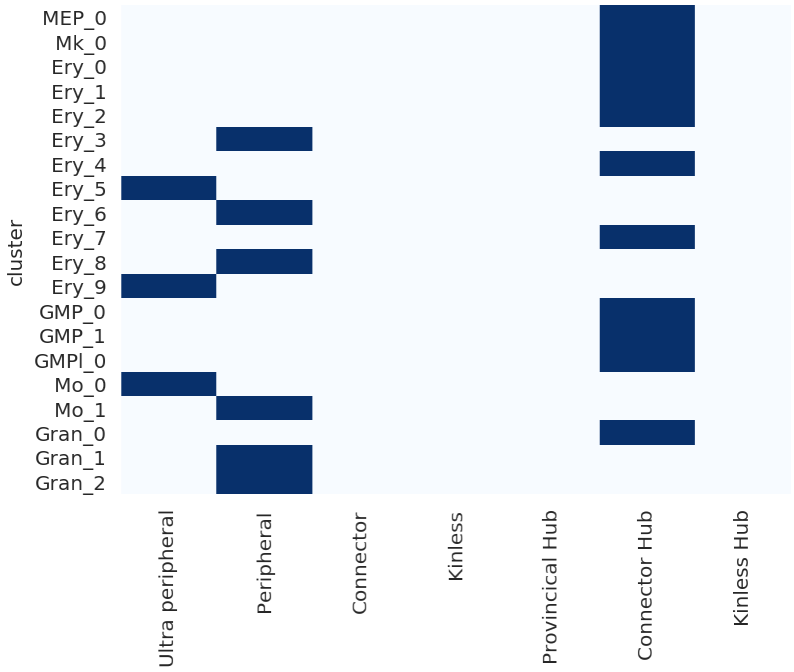



```
Mo_1
```



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

```
Gata2
```



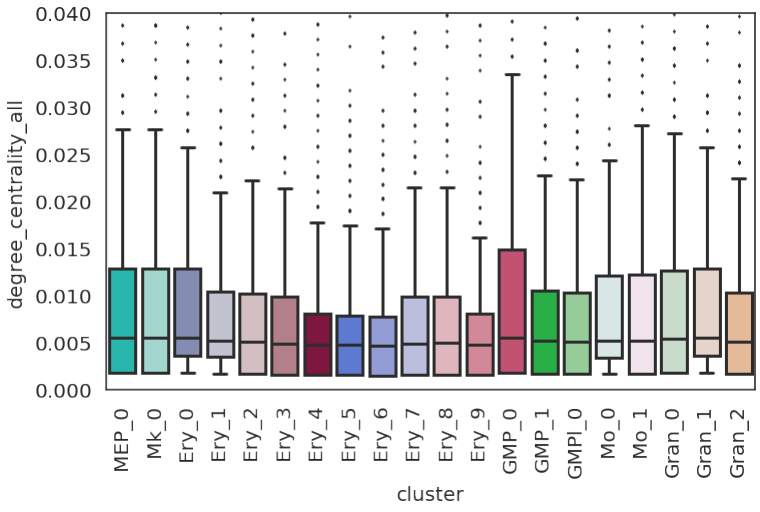
7. Network analysis; Network score distribution

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

7.1. Distribution of network degree

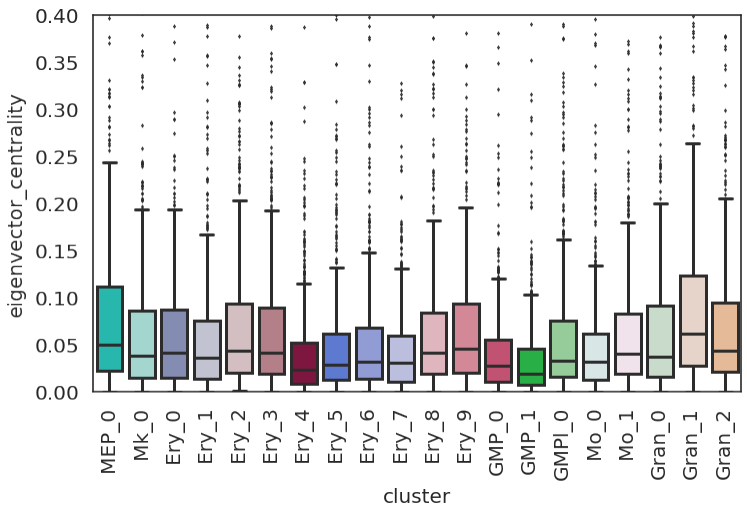
[60]:
```
plt.subplots_adjust(left=0.15, bottom=0.3)
plt.ylim([0,0.040])
links.plot_score_discributions(values=["degree_centrality_all"], method="boxplot",␣
↪save=f"{save_folder}")
```

```
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}")
```

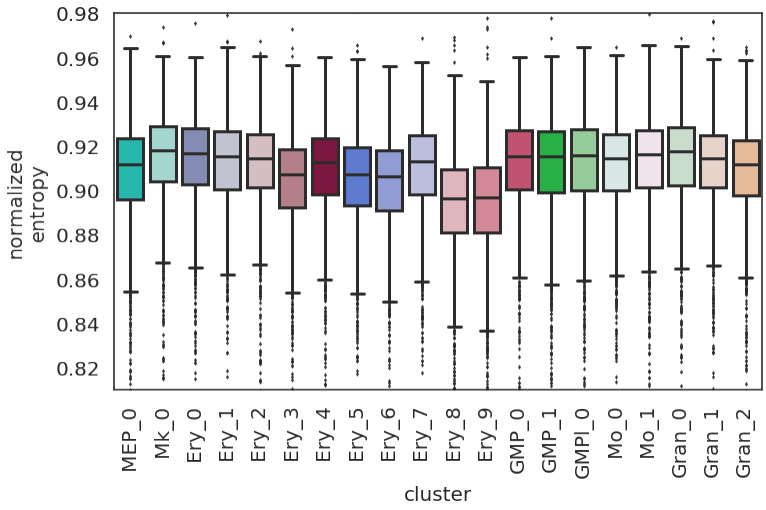
```
eigenvector_centrality
```



7.2. Distribution of netowrk entolopy

[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 score, 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.

In this step, however, we cannot know the specific function or relationship between cell fate.

In the next analysis, we investigate their function and relationship between cell fate by the simulation with GRNs.

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.

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

Besides, we will combine the simulation for the signal propagation and the simulation of cell state transition, which is performed by a python library for RNA-velocity analysis, `velocyto` . A series of simulations visualizes a complex system in which TF controls enormous target genes to determines cell fate.

In short, celloracle provides insight into the regulatory mechanism of cell state from the viewpoint of TF and GRN.

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
```

[3]:
```
import celloracle as co
```

[15]:
```
#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 detil of how to prepare 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 already calculated GRNs. We use this GRNs for simulation.

We import GRNs which was saved in Links object.

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

3. Make predictive models for simulation

We construct ridge regression models again based on the filtered GRN structure. This process takes less time than the GRN inference in the previous notebook because we use only 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
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
```

(continues on next page)

(continued from previous page)

```
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
```

4. in silico Perturbation-simulation

Next, we simulate an effect of perturbation of a TF to investigate the function and regulatory mechanism of the TF.

See the celloracle paper for the details and scientific remnants on the algorithm.

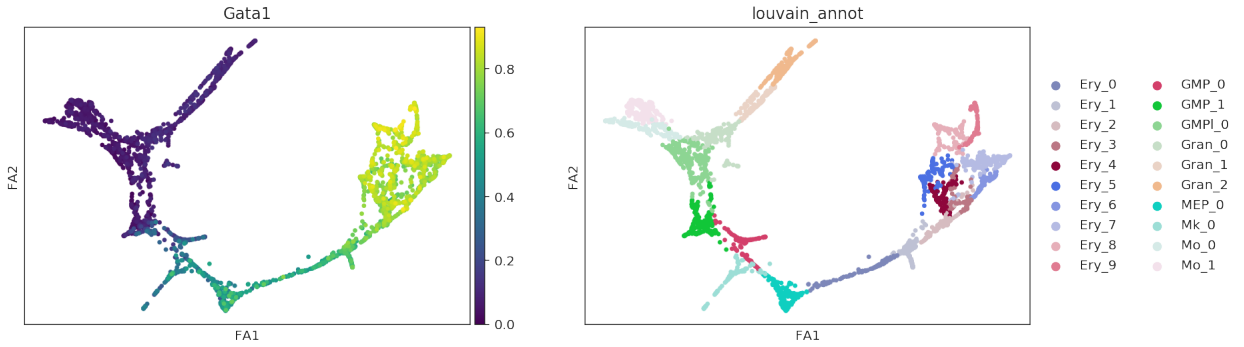
In this notebook, we'll show an example of the simulation; we'll simulate knock-out of Gata1 gene in the hematopoiesis.

Celloracle's simulation supposes to recapitulate previous findings of this gene.

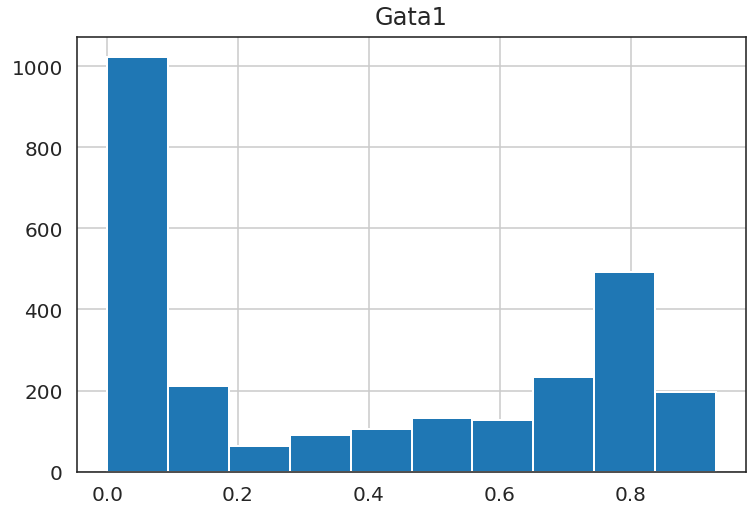
The previous studies have shown that Gata1 is one of the decisive TFs that regulate cell fate in the myeloid progenitors and Gata1 have a positive effect on the erythroid cell differentiation.

4.1. 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()
```



4.1. calculate future gene expression after perturbation.

Although any arbitrary gene expression value can be used for input of perturbation, we recommend avoiding extreme value which is far from natural gene expression range. 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)
```

4.2. calculate transition probability between cells

In the step avobe, we simulated simulated future gene expression after perturbation. This prediction is based on itelative calculations of signal propagations inside the GRN. Here we performed just small number of the calculation, thus the we simulated short future.

Next step, we will calculate the propability of cell state transition based on the simulated data. Using this 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 use some functions imported from velocytoloom class in velocyto.py. Please see the documentation of VelocytoLoom for the detail of parameters in the functions. 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)

# 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.
```

4.3. Visualization

4.3.1. 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)
```

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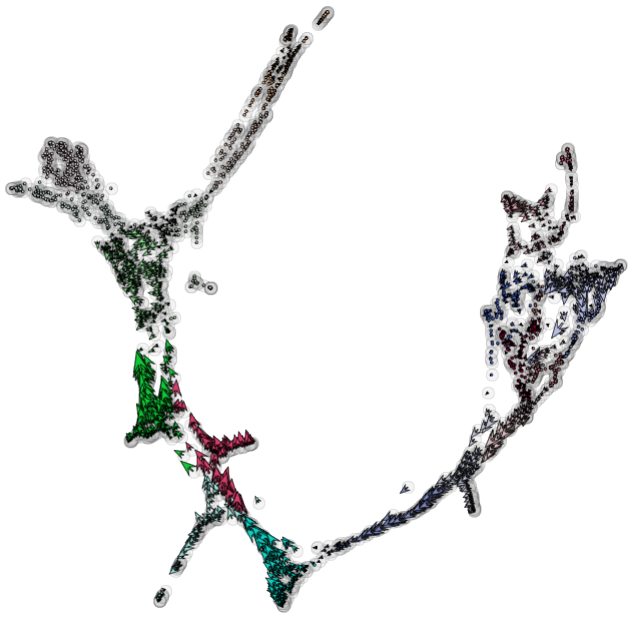
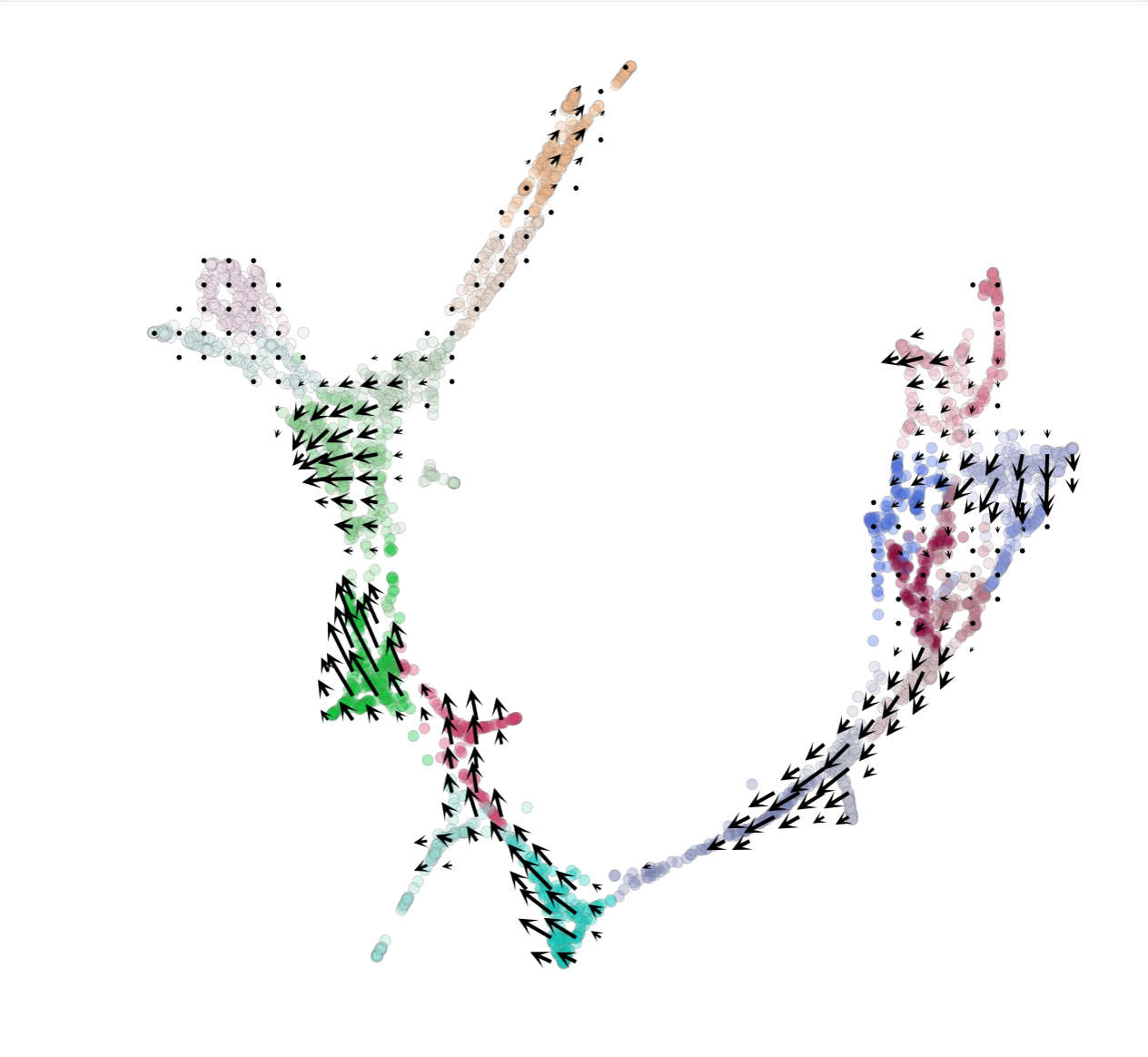
```
quiver_kwargs=dict(headaxislength=7, headlength=11, headwidth=8,
                   linewidths=0.35, width=0.0045,edgecolors="k",
                   color=oracle.colorandum[ix_choice], alpha=1)
plt.quiver(embedding[ix_choice, 0], embedding[ix_choice, 1],
           oracle.delta_embedding[ix_choice,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



4.3.2. 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)
```



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

We can also simulate cell state transition with Markof simulation with the transition probability.

4.4.1. Do simulation

We simulate with the parameters, “n_steps=200” and “n_duplication=5” in the following example.

It means that

1. we will do 200 times of iterative simulations to predict how cell changes over time
2. and we will do 5 round of simulations for each cells.

[83]:
```
%%time
# n_steps is the number of steps in markov simulation.
```

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```
# n_duplication is the number of technical duplicate for the simulation
oracle.run_markov_chain_simulation(n_steps=200, n_duplication=5)
```

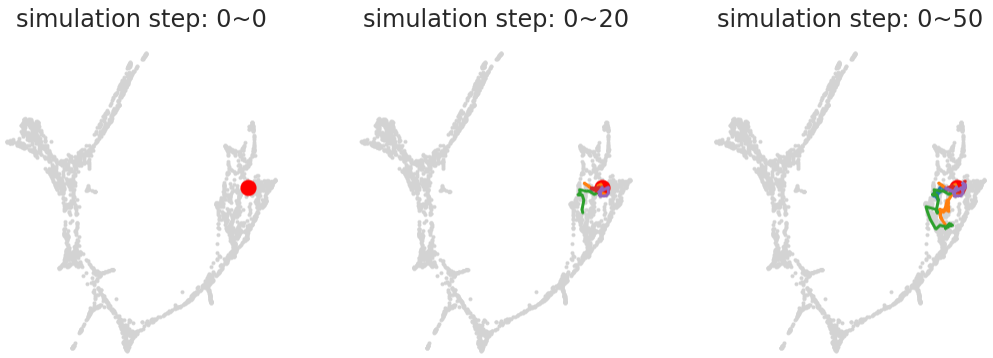
```
CPU times: user 1.33 s, sys: 0 ns, total: 1.33 s
Wall time: 1.33 s
```

4.4.2. Check the results of simulation focusing on a specific cells

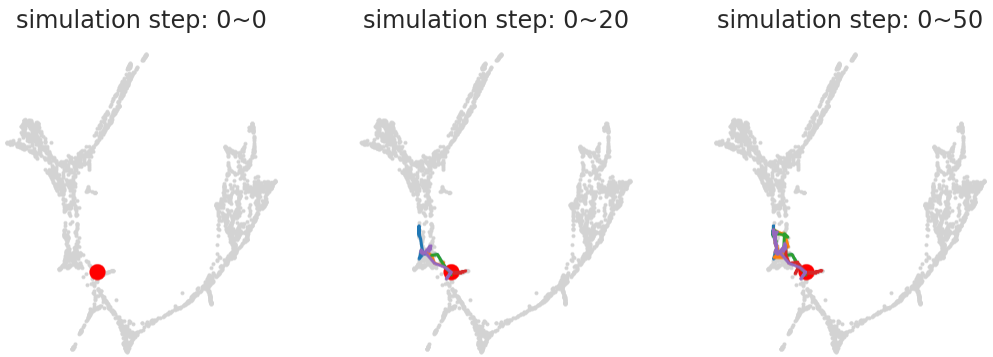
Check the results of simulation. Pick up some cells and visualize their transition trajectory.

[88]:
```
#np.random.seed(12)
# randomly pick up 3 cells
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()
```

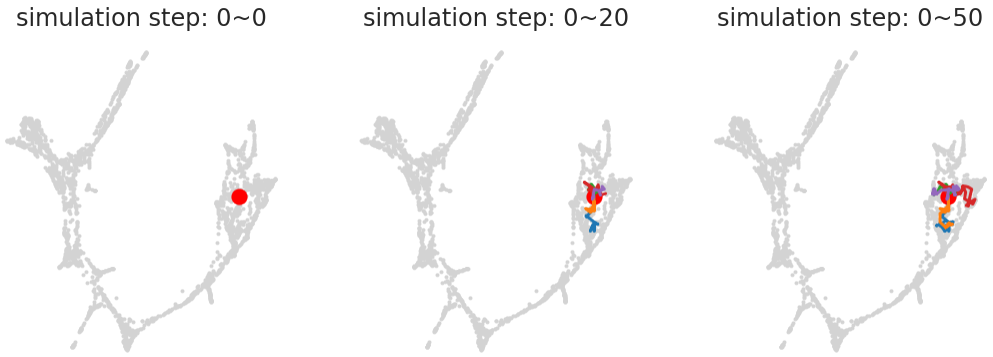
```
cell 1961
```




```
cell 43
```



```
cell 1567
```

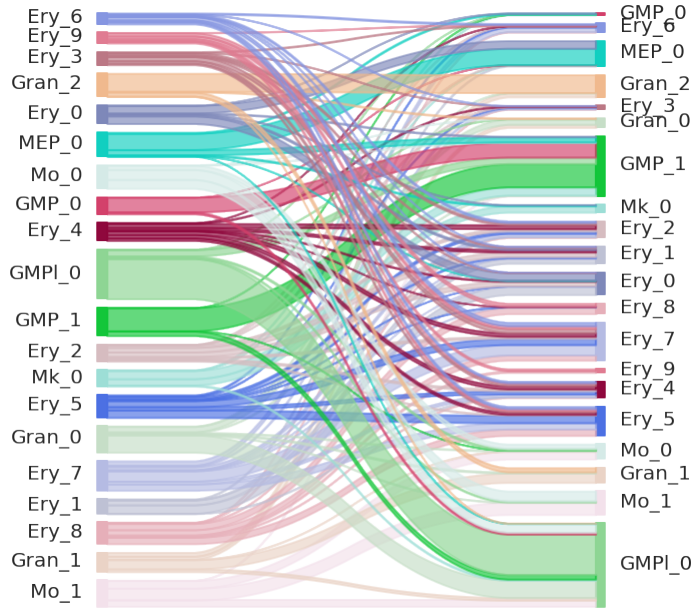


4.4.3. Summarize the results of simulation by plotting sankey diagram

Sankey diagram is useful when you want to visualize cell transition between some groups.

The function below can make a Sankey diagram with any arbitrary cluster unit.

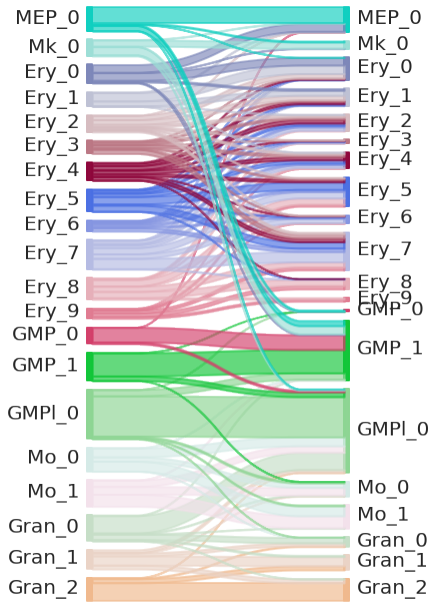
[89]:
```
# plot sankey diagram
plt.figure(figsize=[5,6])
cl = "louvain_annot"
oracle.plot_mc_resutls_as_sankey(cluster_use=cl, start=0, end=100)
```



The sankey diagram above looks messy because cluster order is random.

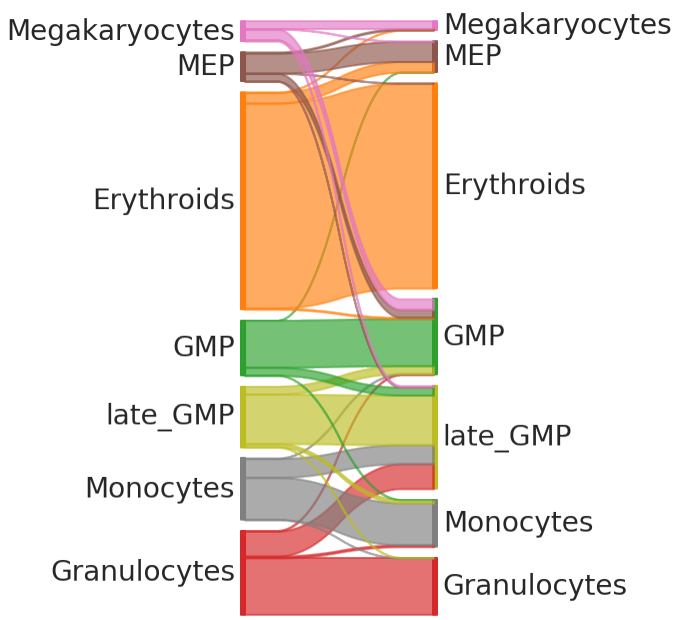
Change cluster order and make plot again

[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 unit.

[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 cell state transition 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 MEP and GMP lineage.
2. Gata1 KO also induced cell state transition from granulocytes to late GMP, suggesting Gata1's involvement in Granulocytes differentiation.

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

[ ]:

1.3 API

1.3.1 Command Line API

CellOracle have 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 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.**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 future gene expression patterns and cell state transition after perturbations of TFs. Please see the paper of CellOracle for details.

The code of the Oracle class was made of three components below.

(1) Anndata: Gene expression matrix and metadata in single-cell RNA-seq are stored in 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 process several data to infer GRN. See the documentation of Net class for detail.

(3) VelycytoLoom: Calculation of transition probability and visualization of directed trajectory graph will be performed in the same way as velocytoloom. VelocytoLoom is class for the Velocyto, which is a python library for RNA-velocity analysis. In the celloracle, we use almost the same functions as velocytoloom for the visualization, but celloracle use simulated gene expression values instead of RNA-velocity data.

Some CellOracle's methods were inspired by velocyto analysis and some codes were made by modifying VelocytoLoom class.

adata
anndata – Imported anndata object

cluster_column_name
str – The column name in adata.obs about cluster info

embedding_name
str – The key name in adata.obsm about dimensional reduction cordinates

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.

fit_GRN_for_simulation(*GRN_unit='cluster'*, *alpha=1*, *use_cluster_specific_TFdict=False*)
Do GRN inference. Please see the paper of CellOracle for details.

GRN can be constructed at an arbitrary cell group. If you want to infer cluster-specific GRN, please set [GRN_unit="cluster"]. GRN will be inferred for each cluster. You can select Cluster information when you import data (not when you run this method.).

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 increase, and you can detect a weak network connection, but it might get more noize. With a higher value of alpha may 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*)

Make 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 increase, and you can detect a weak network connection, but it might get more noize. With a higher value of alpha may reduce the chance of overfitting.
- **bagging_number** (*int*) – The number for 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. See the tutorial of celloracle or motif_analysis module for an example to make such files.

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. Preprocessing (cell and gene filtering, calculate DR and cluster, etc.) should be done before loading data. The method will import NORMALIZED and LOG TRANSFORMED but NOT SCALED and NOT CENTERED DATA. See the tutorial for the details for an example of how to process scRNA-seq data.

Parameters

- **adata** (*anndata*) – anndata object that store scRNA-seq data.
- **cluster_column_name** (*str*) – the column name about cluster info in anndata.obs. Clustering data suppose to be in anndata.obs.
- **embedding_name** (*str*) – the key name about a dimensional reduction 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. Preprocessing (cell and gene filtering, calculate DR and cluster, 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 column name about cluster info in anndata.obs. Clustering data suppose to be in anndata.obs.
- **embedding_name** (*str*) – the key name about a dimensional reduction 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 detail (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 number in markov simulation
- **args** (*dictionary*) – dictionary for the arguments for matplotlib.pyplit.plot. See matplotlib documentation for detail (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 arbitrary 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 sankey-diagram.
- **font_size** (*int*) – Font size for cluster name label in 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*)
Do Markov simlation 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 for multiple times to get a robust outcome.

Parameters

- **n_steps** (*int*) – steps for Markov simulation. This value is equivalent to the time after perturbation.
- **n_duplication** (*int*) – the number for multiple calculations.

simulate_shift (*perturb_condition=None*, *GRN_unit='whole'*, *n_propagation=3*)

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

Three data below are used for the simulation. (1) GRN inference results (coef_matrix). (2) perturb_condition: You can set arbitrary perturbation condition. (3) gene expression matrix: 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 unfeasible values for the perturb condition. It is strongly recommended to check actual gene expression values in your data before selecting perturb condition.
- **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*)

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 existed in the old TF dictionary, old values will be replaced with a new one.

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

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 – Dictionary that store unprocessed network data.

filtered_links

dictionary – Dictionary that store filtered network data.

merged_score

pandas.dataframe – Network scores.

cluster

list of str – List of cluster name.

name
str – Name of clustering unit.

palette
pandas.dataframe – DataFrame that store color information.

add_palette(*cluster_name_list*, *color_list*)
Add color information for each cluster.

Parameters

- **cluster_name_list** (*list of str*) – cluster names.
- **color_list** (*list of stf*) – list of color for each cluster.

filter_links(*p=0.001*, *weight='coef_abs'*, *thread_number=10000*, *genelist_source=None*, *genelist_target=None*)
Filter network edges. In most case inferred GRN has non-significant random edges. We have to remove such random edges before analyzing network structure. You can do the filtering either of three ways below.

1. Do filtering based on the p-value of the network edge. Please enter p-value for thresholding.
2. Do filtering 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 have several types of network weight, so you have to select which network weight do you want to use.
3. Do filtering 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. Please select a type of network weight for the entropy calculation.

Parameters **value** (*str*) – Default is "coef_abs".

get_score(*test_mode=False*)
Get several network sores using R libraries. Make sure all dependant 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 plot of gene network cartography. 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_analisis.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 summary of gene network cartography 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_analisis.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 distribution of network degree (the number of edge per gene). The network degree will be visualized in both linear scale and log scale.

Parameters

- **links** (Links) – See network_analisis.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 of network entropy. See the CellOracle paper for the detail.

Parameters

- **links** (*Links object*) – See network_analisis.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 shows the relationship of a specific network score in two groups.

Parameters

- **links** (Links) – See network_analisis.Links class for detail.
- **value** (*srt*) – The network score type.
- **cluster1** (*str*) – Cluster name. Network scores in the cluster1 will be visualized in the x-axis.
- **cluster2** (*str*) – Cluster name. Network scores in the 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) of GRN in each cluster.

Parameters

- **links** (Links) – See Links class for detail.
- **values** (*list of str*) – The list of score to visualize. If it is None, all network score will be used.
- **method** (*str*) – Plotting method. Select either of "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 of a specific gene between clusters to get an insight into the dynamics of the gene.

Parameters

- **links** (Links) – See network_analisis.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_analisis.Links class for detail.
- **cluster** (*str*) – Cluster nome 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 Oracle class for GRN inference. This class requires two 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 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 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 made from public ATAC-seq dataset of various tissue/cell type.

linkList
pandas.DataFrame – the result of GRN inference.

all_genes
numpy.array – an array of all genes that exist in input gene expression matrix

embedding_name
str – the key name name in adata.obsm about dimensional reduction coordinates

annotation
dictionary – annotation. you can add an arbitrary annotation.

coefs_dict
dictionary – coefs of linear regression.

stats_dict
dictionary – statistic values about coefs.

fitted_genes
list of str – list of genes with which regression model was successfully calculated.

failed_genes
list of str – list of genes that were not able to get coefs

cellstate
pandas.DataFrame – metadata for GRN input

TFinfo
pandas.DataFrame – information about potential regulatory TFs.

gem
pandas.DataFrame – merged matrix made with gene_expression_matrix and cellstate matrix.

gem_standerdized
pandas.DataFrame – almost the same as gem, but the gene_expression_matrix was standardized.

library_last_update_date
str – last update date of this code (this info is for code development. it will be deleted in the future)

object_initiation_date
str – the date when this object is made.

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). The calculation usually takes a long time.

Parameters

- **bagging_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether to scale regulatory gene expression values.
- **model_method** (*str*) – ML model name. "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 to show a progress bar.

fit_All_genes_parallel(*bagging_number=200*, *scaling=True*, *log=None*, *verbose=10*)
!! This function has some bug now and 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 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. The calculation will be performed in parallel using scikit-learn bagging function. You can select a modeling method (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 to scale regulatory gene expression values.
- **model_method** (*str*) – ML model name. "bagging_ridge" or "bayesian_ridge"
- **save_coefs** (*bool*) – Whether 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 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 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 GRN.

Parameters **verbose** (*bool*) – Whether 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.

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.

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.**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.**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.**make_TFinfo_from_scanned_file**(*path_to_raw_bed*, *path_to_scanned_result_bed*, *ref_genome*)
This function is currently an available.

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 in GRN inference.

peak_df
pandas.dataframe – dataframe about DNA peak and target gene data.

all_target_gene
array of str – target genes.

ref_genome
str – reference genome name that was used in DNA peak generation.

scanned_df
dictionary – Results of motif scan. Key is a peak name. Value is a dataframe of motif scan.

dic_targetgene2TFs
dictionary – Final product of Motif scan. Key is a target gene. Value is a list of regulatory candidate genes.

dic_peak2Targetgene
dictionary – Dictionary. Key is a peak name. Value is a list of the target gene.

dic_TF2targetgenes
dictionary – Final product of Motif scan. Key is a TF. Value is a list of potential target genes of the TF.

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 ["individual_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*)
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.

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 tutorial of celloracle documentation.

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

Modules for Network analysis

celloracle.network_analysis module

The *network_analysis* module implements Network analysis.

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 increase, and you can detect a weak network connection, but it might get more noize. With a higher value of alpha may reduce the chance of overfitting.

- **bagging_number** (*int*) – The number for 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.**test_R_libraries_installation**()
CellOracle.network_analysis use some R libraries for network analysis. This is a test function to check instalation of necessary R libraries.

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*

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 – Dictionary that store unprocessed network data.

filtered_links
dictionary – Dictionary that store filtered network data.

merged_score
pandas.dataframe – Network scores.

cluster
list of str – List of cluster name.

name
str – Name of clustering unit.

palette
pandas.dataframe – DataFrame that store color information.

add_palette(*cluster_name_list*, *color_list*)
Add color information for each cluster.

Parameters
- **cluster_name_list** (*list of str*) – cluster names.
- **color_list** (*list of stf*) – list of color for each cluster.

filter_links(*p=0.001*, *weight='coef_abs'*, *thread_number=10000*, *genelist_source=None*, *genelist_target=None*)
Filter network edges. In most case inferred GRN has non-significant random edges. We have to remove such random edges before analyzing network structure. You can do the filtering either of three ways below.
1. Do filtering based on the p-value of the network edge. Please enter p-value for thresholding.
2. Do filtering 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 have several types of network weight, so you have to select which network weight do you want to use.

3. Do filtering 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. Please select a type of network weight for the entropy calculation.

Parameters **value** (*str*) – Default is "coef_abs".

get_score(*test_mode=False*)

Get several network sores using R libraries. Make sure all dependant 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 plot of gene network cartography. 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_analisis.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 summary of gene network cartography 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_analisis.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 distribution of network degree (the number of edge per gene). The network degree will be visualized in both linear scale and log scale.

Parameters

- **links** (Links) – See network_analisis.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 of network entropy. See the CellOracle paper for the detail.

Parameters

- **links** (*Links object*) – See network_analisis.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 shows the relationship of a specific network score in two groups.

Parameters

- **links** (Links) – See network_analisis.Links class for detail.
- **value** (*srt*) – The network score type.
- **cluster1** (*str*) – Cluster name. Network scores in the cluster1 will be visualized in the x-axis.
- **cluster2** (*str*) – Cluster name. Network scores in the 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) of GRN in each cluster.

Parameters

- **links** (Links) – See Links class for detail.
- **values** (*list of str*) – The list of score to visualize. If it is None, all network score will be used.
- **method** (*str*) – Plotting method. Select either of "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 of a specific gene between clusters to get an insight into the dynamics of the gene.

Parameters

- **links** (Links) – See network_analisis.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_analisis.Links class for detail.
- **cluster** (*str*) – Cluster nome 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.**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.

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.**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

Other modules

celloracle.go_analysis module

The *go_analysis* module implements Gene Ontology analysis. This module use goatools internally.

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.**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.**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.

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.**load_pickled_object**(*filepath*)
Load pickled object.
Parameters **filepath** (*str*) – file path.
Returns loaded object.
Return type python object

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.**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 to wait for the process finished. If False, the function finish immediately.
- **return_process** (*bool*) – Whether to return "process".

celloracle.utility.**standard**(*df*)

Standerdize value.

Parameters **df** (*padas.dataframe*) – dataframe.

Returns data after standerdization.

Return type pandas.dataframe

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.**inverse_dictionary**(*dictionary*, *verbose=True*, *return_value_as_numpy=False*)

Make inversed dictionary. See examples below for detail.

Parameters

- **dictionary** (*dict*) – python dictionary
- **verbose** (*bool*) – Whether to show progress bar.
- **return_value_as_numpy** (*bool*) – Whether to convert values into numpy array.

Returns Python dictionary.

Return type dict

Examples

```
>>> dic = {"a": [1, 2, 3], "b": [2, 3, 4]}
>>> inverse_dictionary(dic)
{1: ['a'], 2: ['a', 'b'], 3: ['a', 'b'], 4: ['b']}
```

```
>>> dic = {"a": [1, 2, 3], "b": [2, 3, 4]}
>>> inverse_dictionary(dic, return_value_as_numpy=True)
{1: array(['a'], dtype='<U1'),
 2: array(['a', 'b'], dtype='<U1'),
 3: array(['a', 'b'], dtype='<U1'),
 4: array(['b'], dtype='<U1')}
```

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

1.5 License

The software is provided under xxxxxxx License.

```
License xxxxxxxx

Copyright (c) 2019 Kenji Kamimoto, Christy Hoffmann, Samantha Morris


This is a placeholder. License description will be shown here.
```

1.6 Authors and citations

1.6.1 Cite celloracle

If you use celloracle please cite our bioarxiv preprint TITLE.

1.6.2 celloracle software development

celloracle is developed and maintained by the effort of the members of Samantha Morris Lab. Please post troubles or questions on the Github repository.

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