README

REPTILE

- Regulatory Element Prediction based on TIssue-specific Local Epigenetic marks

https://github.com/yupenghe/REPTILE

REPTILE is a tool to identify the precise location of enhancers by integrating histone modification data and base-resolution DNA methylation profiles.

Please contact Yupeng He for for feedbacks, questions or bugs.

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REQUIREMENT

R

REPTILE requires R (>= 3.2.2) and it is available in R website.

python

REPTILE requires python2 (>= 2.7.9) or python3 (>= 3.5.1). It also requires numpy (>= 1.10.4) and pandas (>= 0.17.1) modules. The numpy and pandas are available from their websties. It is recommended to install anaconda for python2.7 and the two modules will be included.

bedtools

1 - bedtools is available in bedtools website. It is recommended to download and install the latest stable release, according to the installation instruction. Older versions may work but they have not been tested.

2 - After installation, please include the path to the `bedtools` executable in `PATH` environmental variable. The executable is usually in the `bin/` folder within the `bedtools/` folder. Suppose that the path of bedtools executable is `/path/to/bedtools/bin/`, you can add the below command to your `~/.bashrc` file (your shell config file) to accomplish this requirement.

```
export PATH=/path/to/bedtools/bin/:$PATH
```

3 - Please check whether the `bedtools` executable has excecute permission. If not, you will error message "Permission denied". The command below can solve this issue.

```
chmod u+x /path/to/bedtools/bin/bedtools
```

bigWigAverageOverBed

1 - `bigWigAverageOverBed` executable can be downloaded from UCSC genome browser utilities. Search for "bigWigAverageOverBed" under the folder of your operation system. For example, here are the binary releases for linux and

macOS.

2 - After `bigWigAverageOverBed` is downloaded, please run the below command to give it execute permission.

```
chmod u+x bigWigAverageOverBed
```

3 - Last, please include the path to the `bigWigAverageOverBed` executable in `PATH` environmental variable. If the path of executable is `/path/to/bigWigAverageOverBed/` , you can add the below command to `~/.bashrc` file (your shell config file) to accomplish this requirement.

```
export PATH=/path/to/bigWigAverageOverBed/:$PATH
```

INSTALLATION

1 - REPTILE can be downloaded (cloned) using git command.

```
git clone https://github.com/yupenghe/REPTILE.git
```

2 - The R library required for REPTILE can be installed through CRAN by command below.

```
R
> install.packages("REPTILE")
```

More information about this R package is available in REPTILE CRAN webpage.

3 - To make REPTILE easier to use, it is recommended to add the path of executable scripts (in the `bin/` folder) from REPTILE in `PATH` environmental variable Otherwise, the path of the scripts has to be typed for each use. Suppose the path is `/path/to/REPTILE/bin/` , please add the below command to `~/.bashrc` file (your shell config file).

```
export PATH=/path/to/REPTILE/bin/:$PATH
```

UNINSTALLATION

To uninstall REPTILE, please run the below command to remove the installed R package. You may want to remove the REPTILE folder and related files to fully clean up.

```
R CMD UNINSTALL REPTILE
```

TEST REPTILE

Below command can be used to test whether REPTILE is correctly installed and all requirements are met.

```
cd REPTILE/test
./run_test.py
```

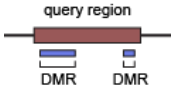
REPTILE OVERVIEW

Key concepts

REPTILE is a tool to identify enhancers by epigenomic data (DNA methylation and histone modifications). There two key concepts in REPTILE, "query region" and "DMR":

- query region - (training) known enhancers and regions with no observable enhancer activity. (prediction) sliding genomic windowns used to call the enhancers that show little methylation variation (and thus contain no DMR).

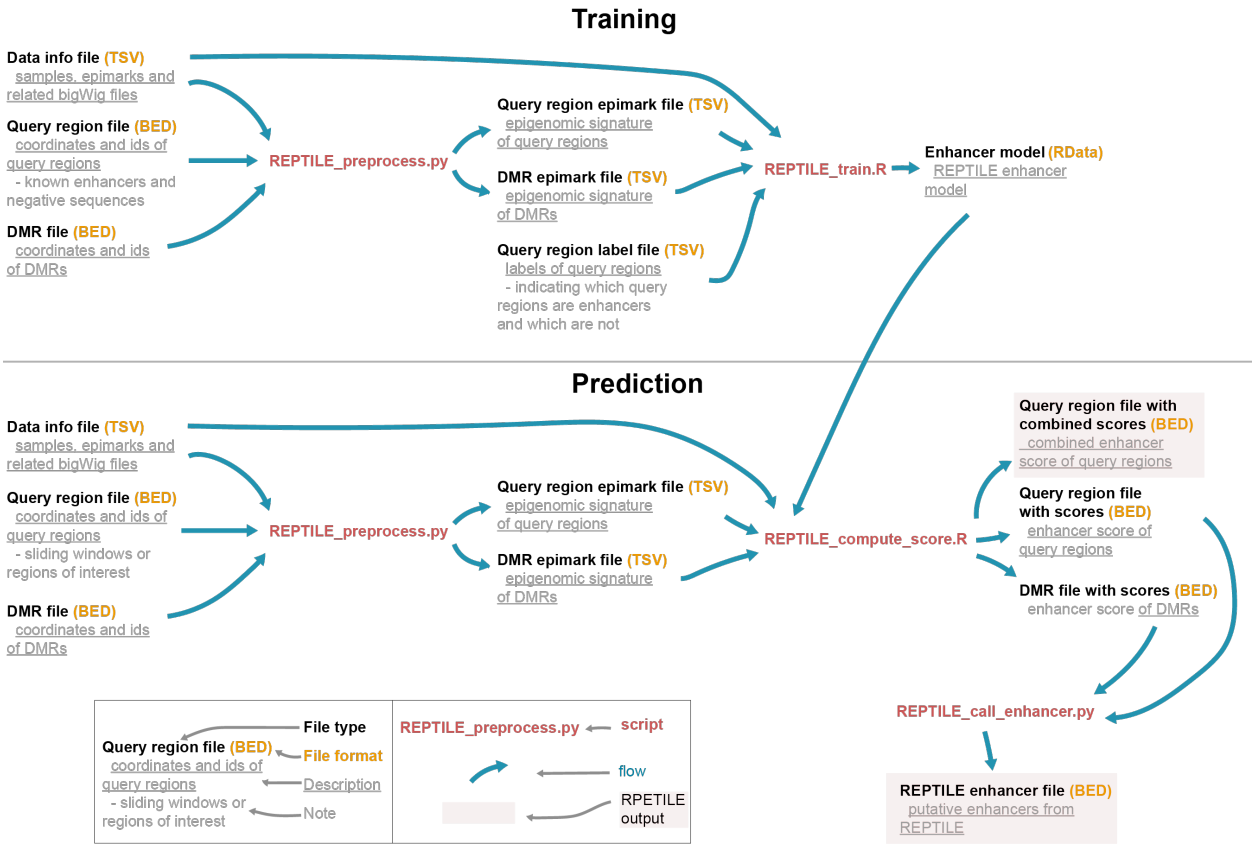
- DMR - differentially methylated regions, used as high-resolution enhancer candidates



The currently known enhancers (~2kb) are generally much larger than the binding motifs of TFs (~10-20bp) and are likely to include sequences that contribute little to their enhancer activity. We used the term “query region” to describe such large regions where a small fraction of the region is regulatory (if any). Query region also refers to negative regions (that showed no observable enhancer activity) and the genomic windows used by enhancer prediction methods. Since large portion of an active query region is likely to have little contribution to its enhancer activity, the epigenomic signature of the whole active query region may not be a good approximation to the epigenomic state of the bona fide regulatory sequences within it. To address this issue, we used differentially methylated regions (DMRs, ~500bp) to pinpoint the possible regulatory sub-regions within query regions. DMRs serve as high-resolution enhancer candidates and may capture the local epigenomic patterns that would otherwise be averaged/washed out in analysis focusing on the query regions.

REPTILE workflow

The workflow of REPTILE pipeline:



Check File format section for details of each file.

USE REPTILE

As shown in the REPTILE workflow, REPTILE pipeline can be used to 1) learn enhancer models from the epigenomic signature of known enhancers and negative sequences and 2) then generate enhancer prediction based on the model. The pipeline is composed by five executable scripts (in `bin/`). `REPTILE_train.R` and `REPTILE_compute_score.R` are the key scripts of doing training and generate prediction, respectively. The inputs for these scripts can be readily generated using `REPTILE_preprocess.py`. `REPTILE_call_enhancer.py` is used to generate enhancer calls based on the results (enhancer scores) from `REPTILE_compute_score.R`. The enhancer predictions can be evaluated using the last script, `REPTILE_evaluate_prediction.R`.

The whole RPETILE pipeline can be divided in the several steps. First, input files (in commonly used file formats of genomic data) are preprocessed to generate the inputs for following training and prediction. Next, in training step, REPTILE learns an enhancer model. The model will then be used to calculate enhancer scores for each query region and DMR in the prediction step. Lastly, enhancer calls will be generated based on these scores.

Calling DMRs

DMRs can improve the resolution of REPTILE predictions but DMR calling is not part of REPTILE pipeline. Several published methods are available to call DMRs, including bsseq, MOABS and DSS. The DMRs in the example data were called by methypy. It is a python module that is designed to identify DMRs across a large number of cells and tissues, whereas existing methods are better at doing pairwise comparison.

The DMR input is optional and REPTILE still generates good predictions without it. If you want to run REPTILE without DMR input, ignoring `-d` option when running RPETILE scripts will do.

Preprocessing

The goal of preprocessing step is to generate the files of epigenomic signatures of DMRs and query regions. These files are inputs for follow-up training and prediction steps.

```
REPTILE_preprocess.py \
      data_info_file \
      query_region_file \
      -d dmr_file \
      output_prefix \
      -p num_processors
```

Note that for generating genome-wide predictions, `-g` option should be used. This option should not be enabled in the preparation of inputs for training or the calculation of enhancer scores for given regions (i.e. `REPTILE_compute_score.R` with `-w` option). `REPTILE_preprocess.py -h` to get help information.

Training an enhancer model

In training step, an enhancer model is learned based on the epigenomic signatures of known enhancers and negative sequences (`query_region_epimark_file` and `dmr_epimark_file`) generated in preprocessing step. The file `query_region_label_file` specifies the labels of query regions: which regions are enhancers and which region are negative sequencers.

```
REPTILE_train.R \
   -i data_info_file \
   -a query_region_epimark_file \
   -d dmr_epimark_file \
   -l query_region_label_file \
   -s training_sample_name \
   -o output_prefix
```

`REPTILE_train.R -h` to get help information.

Generate enhancer scores

Enhancer (confidence) scores are calculated for DMRs and query regions.

```
REPTILE_compute_score.R \
   -i data_info_file \
   -m enhancer_model \
   -a query_region_epimark_file \
   -d dmr_epimark_file \
   -s prediction_sample_name \
   -o output_prefix
```

For generating enhancer scores of given regions, option `-w` should be used and in preprocessing the inputs, `-g` should NOT be used (in running `REPTILE_preprocess.py`). If `-w` is used, the combined score will also be calculated. The combined score for each query region is the maximum of scores of the query region and DMRs overlapping with it. `REPTILE_compute_score.R -h` to get help information.

Get enhancer calls

Based on the enhancer scores of DMRs and query regions, putative enhancers can be generated.

```
REPTILE_call_enhancers.py \
    query_region_file_with_score \
    -d dmr_file_with_score \
    -o output_file_name \
    -p score_cutoff
```

For genome-wide prediction, it is recommend to use sliding genomic windows as query regions. `REPTILE_call_enhancers.py -h` to get help information.

Evaluating enhancer prediction results

The area under the receiver operating characteristic curve (AUROC), area under the precision-recall curve (AUPR) and other metrics will be calculated for users to evaluate the prediction accuracy.

```
REPTILE_evaluate_prediction.R
    -p query_region_file_with_combined_score \
    -l query_region_label_file \
    -s target_sample
```

`target_sample` is the sample in which enhancer scores are calculated. `REPTILE_evaluate_prediction.R -h` to get help information.

EXAMPLE

Please see EXAMPLE.md for details. The simple example can be run with code below. 1 Gb hard drive space and 1 Gb memory are needed to run it.

```
cd simple_example/
sh run_simple_example.sh > log 2> err
```

Code below can be used to run the complete example. Please be aware that running the complete example requires large memory and check with the requirement first.

```
cd example/
sh run_example.sh > log 2> err
```

PRETRAINED MODEL

Please see PRETRAINED_MODEL.md for details.

FILE FORMAT

The formats of files invovled in the REPTILE workflow are:

Data info file (TSV)

Tab-separated file providing information about samples, epigenetic marks and path to corresponding bigWig files. No duplicated mark name is allowed for each sample and no duplicated sample name is allowed for each mark. It contains a header showing the exactly three column names, “sample”, “mark” and “bw_file”.

Format (with header):
<sample name><\t><mark name><\t><path to bigwig file>

```
sample    mark      bw_file
tissue1   mCG       /path/to/bw/tissue1_Meth.bw
tissue2   mCG       /path/to/bw/tissue2_Meth.bw
tissue1   H3K27ac   /path/to/bw/tissue1_H3K27ac.bw
tissue2   H3K27ac   /path/to/bw/tissue2_H3K27ac.bw
...
```

Query region file (BED)
DMR file (BED)

BED file of query regions or DMRs.

Format (no header):
<chromosome><\t><start><\t><end><\t><id>

```
chr3    137783219 137786437    enhc40
chr13   105537275 105539272    enhc41
chr12   104939330 104940069    neg123
...
```

Query region label file (TSV)

Tab-separated file labeling what annotated regions are active in which sample(s).

Format (with header):
First line is a header indicating the content of each column. Name of first column is "id" and others are sample names. The file has multiple columns. First column is region id. Each following column corresponds to one sample and the values indicate whether the region is active in the sample:
- 1: active,
- 0: no activity
- NA: unknown

```
id        tissue1   tissue2
enhc40    1         1
enhc41    0         1
neg123    0         0
...
```

Query region epimark file (TSV)
DMR epimark file (TSV)

Direct output from “REPTILE_preprocess.py”.
Tab-separated file of epigenetic profiles of query regions or DMRs

Format (with header):
First line is a header indicating the content of each column. First four columns are chromosome, start, end and id. Following columns are the scores/enrichment of epigenetic marks.

```
chr     start      end        id      mCG_tissue1 ...
chr3    137783219  137786437  enhc40  0.56 ...
chr13   105537275  105539272  enhc41  0.73 ...
chr12   104939330  104940069  neg123  0.98 ...
...
```

Query region file with combined scores (BED)
Query region file with scores (BED)
DMR file with scores (BED)
REPTILE enhancer file (BED)

BED file of query regions, DMRs or enhancer calls with enhancer scores.

Format:
<chromosome><\t><start><\t><end><\t><id><\t><score>

```
chr3    137783219 137786437    enhc40   0.94
chr13   105537275 105539272    enhc41   0.78
chr12   104939330 104940069    neg123   0.23
...
```

Enhancer model (RData)

Enhancer model learned by REPTILE. Direct output from “REPTILE_train.R”.

Format:
R data. The file includes a variable, which is list containing two objects of class randomForest:
D - Classifier for DMRs. It is an randomForest object or glm object when family
is set to be "Logistic".
R - Classifier for query regions. It is an randomForest object or glm object when family is set to be "Logistic".