PING: Probabilistic Inference for Nucleosome Positioning with MNase-based or Sonicated Short-read Data.

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This vignette presents a workflow to use PING on paired-end sequencing data.

Contents

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1 Licensing and citing

Under the Artistic License 2.0, you are free to use and redistribute this software.

If you use this package for a publication, we would ask you to cite the following:

> Xuekui Zhang, Gordon Robertson, Sangsoon Woo, Brad G. Hoffman, and Raphael Gottardo. (2012). Probabilistic Inference for Nucleosome Positioning with MNase-based or Sonicated Short-read Data. PLoS ONE 7(2): e32095.

2 Introduction

For an introduction to the biological background and PING method, please refer to the PING user guide.

3 PING analysis steps

A typical PING analysis consists of the following steps:

1. Extract reads and chromosomes from bam files.

2. Segment the genome into candidate regions that have sufficient aligned reads via 'segmentPING'

3. Estimate nucleosome positions and other parameters with PING

4. Post-process PING predictions to correct certain predictions

As with any R package, you should first load it with the following command:

```
> library(PING)
```

4 Data Input and Formatting

In order to use the PE version of PING, the input has to be slightly different. Instead of a GRanges object, the new segmentation method use a list of reads and a chromosome.

From a bed file, you can create such a list manually by first reading the file using read.table, then assigning the resulting data.frame to the 'P' attribute of a list. If some reads are missing an end or a start coordinate, they can still be used. The reads with a missing start are treated as reverse reads and should be assigned to an attribute 'yRm' to the same list. Reads with a missing end are treated as Forward reads and have to be assigned to an attribute 'yFm'.

We provide a dataset for the chromosome M of yeast.

```
> data(yeast_chrM)
> head(yeast_chrM$P)

                  qname pos.- pos.+
4059237   120:6253:2074   338   187
4059238   42:9052:11042   313   194
4059239   17:6495:10151   341   209
4059240    81:14542:7245  341   209
4059241  87:14926:13898   341   209
4059242 101:5324:18045    341   209
```

5 PING analysis

5.1 Genome segmentation

PING is used the same way for paired-end and single-end sequencing data. The function segmentPING will decide which segmentation method should be used based on the data type. When dealing with paired-end data, four new arguments have to be passed to the function: a chromosome chr and three parameters used in candidate region selection: islandDepth, min_cut and max_cut.

These arguments control the size and required coverage for a region to be considered as a candidate.

In order to improve the computational efficiency of the PING package, if you have access to multiple cores we recommend that you do parallel computations via the parallel package. In what follows, we assume that parallel is installed on your machine. If it is not, you could omit the first line, and calculations will occur on a single CPU. By default the command is not run. Note that the segmentPING and PING functions will automatically detect whether you have initialized a cluster and will use it if you have.

```
> library(parallel)
```

```
> segPE <- segmentPING(yeast_chrM, chr = "chrM", islandDepth = 3,
      min_cut = 50, max_cut = 1000)
```

It returns a `segReadsListPE` object.

5.2 Parameter estimation

The only difference when using `PING` for paired-end data is the argument
PE that has to be set to TRUE.

```
> ping <- PING(segPE, PE = TRUE)
```

The returned object is of class pingList and can be post-processed.

6 Post-processing PING results

Here again, we set the argument PE to TRUE, and use postPING normally.

```
> {
      sigmaB2 = 3600
      rho2 = 15
      alpha2 = 98
      beta2 = 2e+05
 }
> PS = postPING(ping, segPE, rho2 = rho2, alpha2 = alpha2, beta2 = beta2,
      sigmaB2 = sigmaB2, PE = TRUE)

 The 6 Regions with following IDs are reprocessed for singularity problem:
(0.773,114]80    (114,228]39    (114,228]51    (114,228]79    (114,228]82
           80             153            165            193            196
 (114,228]106
          220


 The 17 Regions with following IDs are reprocessed for atypical delta:
[1] 155 190 129   41   37   28
[1] "No predictions with atypical sigma"

 The 172 regions with following IDs are reprocessed for Boundary problems:
[1]   4   6   7 12 18 20
```

The result output PS is a dataframe that contains estimated parameters of each nucleosome, users can use write.table command to export the selected columns of the result.

```
> head(PS)
```

```
      ID  chr          w          mu    delta sigmaSqF  sigmaSqR          se
96    32 chrM 0.3593627  11633.3048 119.5366  876.2446   925.5663 12.894380
518  163 chrM 0.6357604  63067.6607 139.9552 1377.8030  1047.7658  5.252400
1      1 chrM 0.5649540    334.6209 135.6721 1224.5633  1536.3315  8.075665
62    21 chrM 0.3226948   7231.8196 171.9921 1509.9668  1268.8164 10.375752
700  228 chrM 0.3263701  84916.4188 149.8338  955.7443  1378.1164  8.668972
524  164 chrM 0.4261601  64274.6381 141.5827 2071.6632  1815.6545 13.725905
         score    scoreF    scoreR minRange maxRange       seF       seR rank
96   1217064.4 628162.3 588902.1    11290    12500 15.98384 11.486267    1
518  1036712.8 518356.4 518356.4    62728    63762  7.37211  6.219118    2
1     955330.1 510381.8 444948.3      187      949  9.26240  9.202644    3
62    929156.7 405688.1 523468.6     6838     8133 11.68977 11.680681    4
700   920082.6 531315.3 388767.3    84565    85803 11.57234  9.151415    5
524   894164.8 375808.4 518356.4    63281    64582 15.56970 14.943394    6
```

7   Using the results

PING comes with a set of tools to export or visualize the prediction. Here, we only show how to make a quick plot to summarize the prediction. For more information on how to export the results or make more complex plots, refer to the section 'Result output' of PING vignette.

The function plotSummary will generate a plot displaying the coverage by the reads used as input ($yeast_chrM$) and the predicted position of the nucleosomes of PS for the given ranges.

```
> plotSummary(PS, yeast_chrM, chr = "chrM", from = 1000, to = 4000)
```

PING\unhbox \voidb@x \penalty \@M \hskip \z@skip \T1\textunderscore

_PE-plotSummary.png