Using PING with Paired-End sequencing data

Xuekui Zhang[*], Sangsoon Woo[†], Raphael Gottardo[‡] and Renan Sauteraud[§]

November 9, 2012

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

Contents

[*] ubcxzhang@gmail.com
[†] swoo@fhcrc.org
[‡] rgottard@fhcrc.org
[§] rsautera@fhcrc.org

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 other vignette: 'The `PING` user guide'. Because the structure of paired-end sequencing data requires a slightly different treatment , we are separately presenting how to use `PING` for these data in this vignette.

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

As with the Single-End `PING`, the input used for the segmentation step is a `GRanges` object.

Because Paired-End sequencing data often comes in the form of BAM files, we provide a function called `bam2gr` to convert these files into `GRanges` objects with all the appropriate information. A small BAM file including two chromosomes of the yeast is provided to be used as an example in this vignette.

```
> yeastBam <- system.file("extdata/yeastChrI_M.bam", package = "PING")

> gr <- bam2gr(bamFile = yeastBam, PE = TRUE)

Chromosome  chrI
Chromosome  chrM
```

gr is a `GRanges` object containing all the reads from the .bam file.

Note that this function will also work for single-end sequencing data and the argument `PE` should be set to TRUE when dealing with paired-end data.

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 arguments provided. When dealing with paired-end data, four new arguments have to be passed to the function: `islandDepth`, `min_cut` and `max_cut` for candidate region selection. These arguments control the size and required coverage for a region to be considered as a candidate.

Parallelisation will also work with paired-end data. In what follows, we assume that `parallel` is installed on your machine. If it is not, the first line should be omitted and calculations will occur on a single CPU.

```
> library(parallel)
```

In order to run `segmentPING`, we have to subset our GRanges object to have a single chromosome

```
> grM <- gr[seqnames(gr) == "chrM"]
> seqlevels(grM) <- "chrM"

> segPE <- segmentPING(grM, PE = TRUE)
```

It returns a `segReadsListPE` object.

5.2 Parameter estimation

```
> ping <- PING(segPE, nCores = 2)
```

The returned object is a `pingList`, which will go through a post-processing step using `postPING` function.

6 Post-processing PING results

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

 The 5 Regions with following IDs are reprocessed for singularity problem:
[1]  84 146 159 185 188

 The 1 Regions with following IDs are reprocessed for atypical delta:
[1] 149
[1] "No predictions with atypical sigma"

 The 146 regions with following IDs are reprocessed for Boundary problems:
[1]  4 17 25 37 39 46
```

The result output of `postPING` is a dataframe that contains estimated parameters of each nucleosome.

7 Analyzing the prediction

`PING` comes with a set of tools to export or visualize the prediction. Here, we only show how to export the results into bed format for further analysis and how to make a quick plot to summarize the nucleosome prediction. For more information on how to export the results or make more complex figures, please refer to the section 'Result output' of PING vignette.

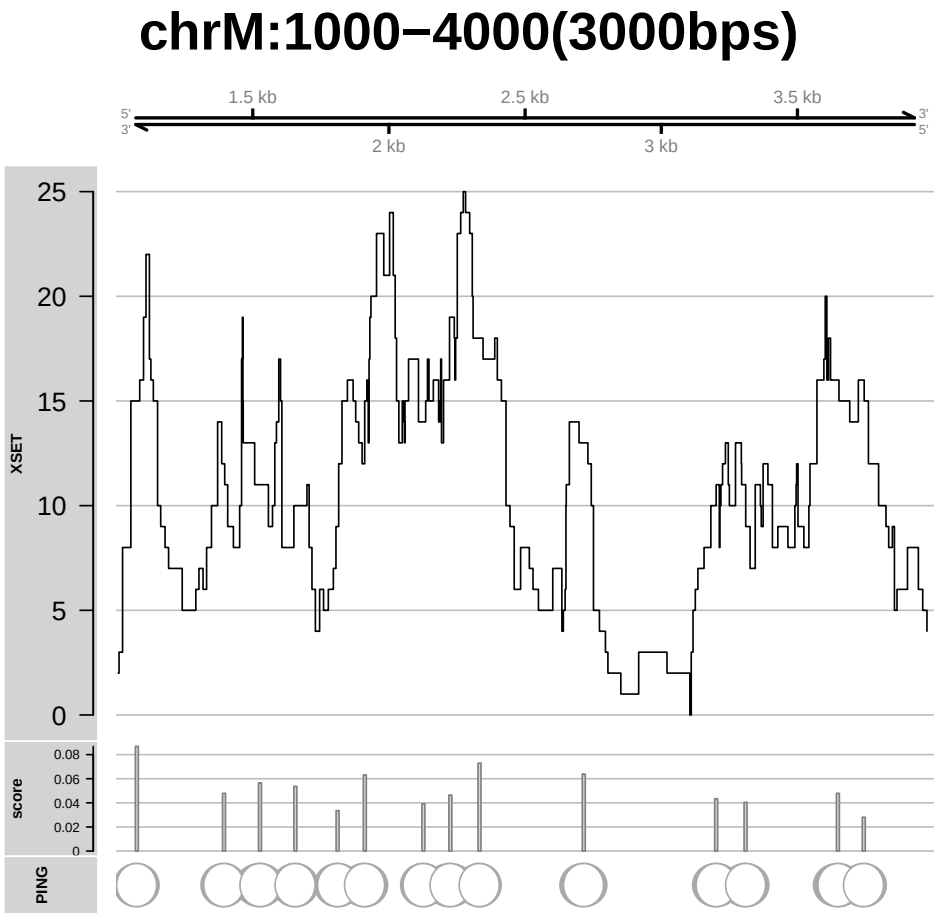
The function `makeRangedDataOutput` offers a simple way to convert the prediction results into a `RangedData` objec that can be exported into a file using the `rtracklayer` package.

```
> rdBed <- makeRangedDataOutput(PS, type = "bed")
> library(rtracklayer)
> export(rdBed, "nucPrediction.bed")
```

The exported file includes all information about the predicted nucleosomes, which are already automatically ranked by their score.

For paired-end sequencing data, the bult-in plotting function `plotSummary` can be used to visualize the predicted nucleosome positions obtained from `postPING` function.

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



All the arguments for this function will work for Paired-end data as well. Refer to PING vignette and the man page ?plotSummary for more information.