**Genetic Location Identification via Bionano**

**Version 1.6**

**User manual**

**Chunlong Chen, Weitao Wang**

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**Contents**

Prerequisites

1. JDK version 1.8
2. R is required for peak calling
3. About 7GB of available RAM
4. Steps in this document assumes that your current working directory is ‘example’ and that the ‘replicon’ executable is in the parent directory.

Introduction

This is an extension software analyzing data based on Bionano high throughput single‑molecule imaging platform determination of any biological process associated with fluorescently labeled DNA, for example: DNA replication, DNA recombination, DNA repair. It provides the analysis on each single molecule as well as the frequency occurred in population‑based data along the genome and the given genomic positions of interest.

Packages

Notice: Please pay attention that the parent directories should be the folder containing all corresponding files sharing the same filename (indicated by the “SampleName” parameter) besides suffix, i.e. the parent directories containing all the .bnx, .xmap, .rcmap, .qcmap or .txt input files and output files must be with the same file name beside suffix, and must be end with corresponding suffix for data format recognition.

1. AllRawDataRefining\_1G2R.jar

The input data are 4 files with basial format of Bionano platform: they are [bnx](https://bionanogenomics.com/wp-content/uploads/2018/04/30038-BNX-File-Format-Specification-Sheet.pdf) (raw labeling signal data) , [xmap](https://bionanogenomics.com/wp-content/uploads/2017/03/30040-XMAP-File-Format-Specification-Sheet.pdf) (mapping data), qcmap (base calibration data) ,rcmap (genome reference data). The jar packages should be run under terminal. It will refine all information from these 4 input files, filter fibers and calculate the precise genomic positions of labelling signals on each filtered fiber. Normally, there are 2 channels, one stores the signals (green in our case) for mapping fibers to reference, another is used to store the labelling the signals of interest (in our case, replication signals in red). Different experimental designs/settings may have different choices for which channels to use. By default, the current package uses channel 2 as labeling channel of interest, which is most comment setting. For the other case, i.e. using channel 1 for signals of interest, and channel 2 for mapping, please use instead the package AllRawDataRefining\_1R2G.jar.

**Parameters**:

-B: BNX parent directory

-X: XMP parent directory

-R: RCmap parent directory

-Q: QCmap parent directory

-O: Output directory

-SampleName: All SampleName seperated by "," delimiter, same as all filenames in various parent paths and without any suffix

**An Example (Quick start):**

java -jar AllRawDataRefining\_1G2R.jar

-B /Volumes/WWT/Final-Version/1905/BNX/

-X /Volumes/WWT/Final-Version/1905/XMP/

-R /Volumes/WWT/Final-Version/1905/RCmap/

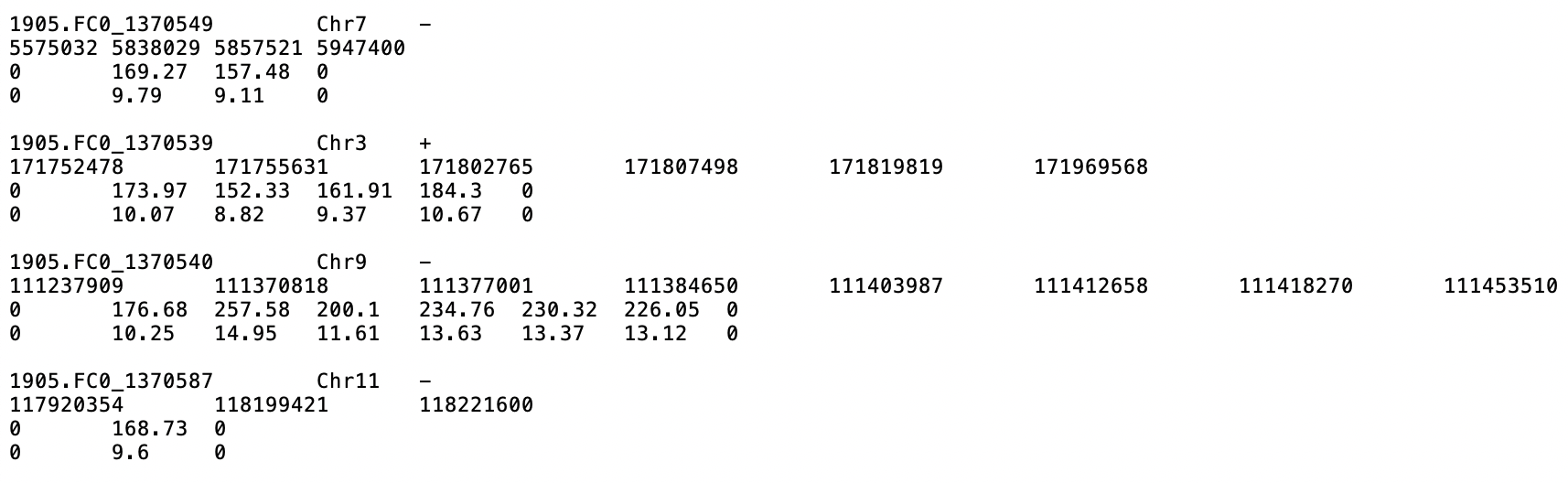
-Q /Volumes/WWT/Final-Version/1905/QCmap/

-O /Volumes/WWT/Final-Version/1905/

-SampleName 1905async,1905.FC0

**The output files**

A .txt file records all refined calculation results about mapped fibers with signals of interest (in our case, ORM signals in red channel) after base calibration from raw data (qcmap,rcmap, xmp and bnx files). An example is shown below. Most downstream analyses use it as input file.

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Each fiber record is organized by 4 lines. The 1st line gives the record ID (the 1st item) organized by Sample name and molecular ID from .bnx file separated by “\_”, the chromosome (the 2nd item) and the strand (the 3rd item) where the fiber maps to. The second line is the genomic positions of all labeling signals and the coordinate of 2 ends of fiber (the first position and last position). The 3rd and 4th line are the corresponding SNR (signal noise ratio) and signal intensity of each corresponding signal from .bnx file.

AllRawDataRefining\_1R2G.jar

The same parameters as AllRawDataRefining\_1G2R, and the only difference is that, in AllRawDataRefining\_1R2G.jar, the channel 1 is used for labelling signals of interest and channel 2 is used as mapping signals.

1. GenerateGTF\_ByAllDataRefining\_Reformat.jar

The input data needs .txt file(s) got by AllRawDataRefining\_1R2G.jar or AllRawDataRefining\_1G2R.jar. It is used to generate a gtf-like file for each input .txt file, in order to be able to visualize the fibers together with their labelling signals on the IGV (or any other genomic browsers that can load gtf file).

Parameters:

-I: The parent path of the input txt file(s)

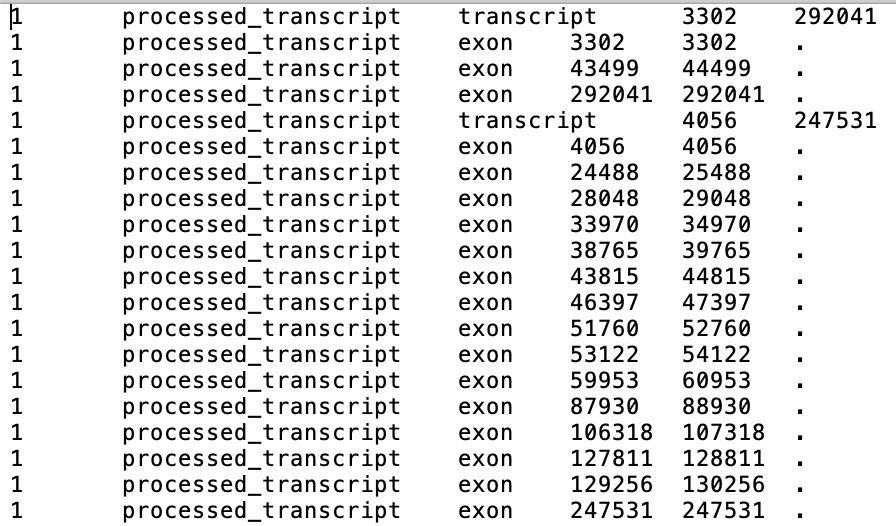
-O: The path of output gtf file(s)

-SampleName: the name of all txt file, multi files will be separated by “,” and the name of txt file can’t contain character “\_”, since it has been used as delimiter in script.

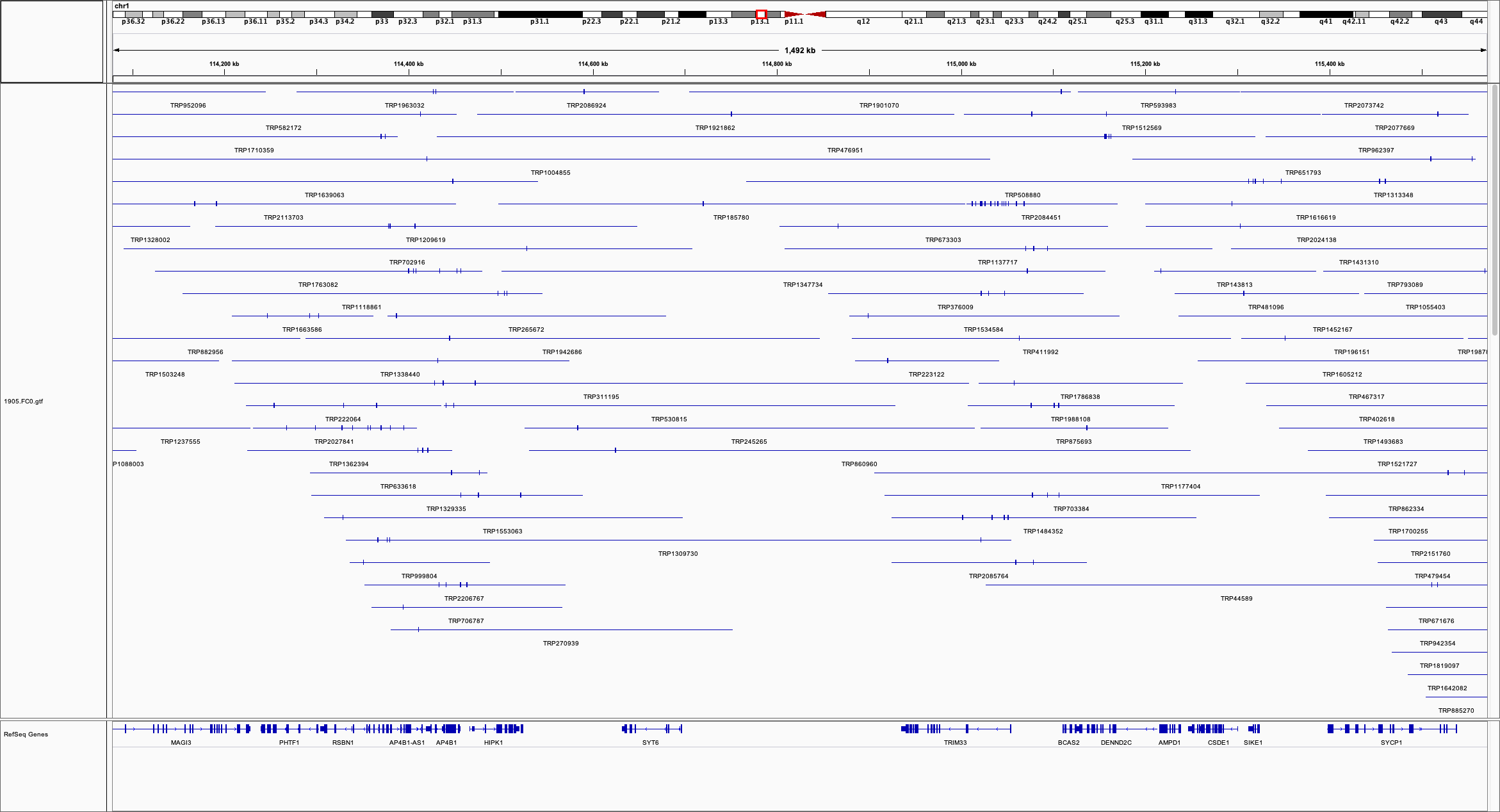
-Contain: The option could only be T when it shows up, and the default value is “false” when it is missing. It is used to filter out the fibers without any signal of interest, default is unfiltering.

**The output files**

A gtf-like file (.gtf) for each input .txt file.

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Here, each fiber is labeled as a transcript and for data visualization in IGV. We labeled all labelling signals and 2 ends of each fiber as exons. The start and end coordinates for the start and end positions, respectively, of each fiber is identical (i.e. exon length equal to 1 nt). For the visualization reason, the start and end coordinates of each signal is set as its position – and +, respectively, 500 nt (i.e. exon length equal to 1 kb). As illustrated in the following figure, each line is a fiber and each little bar on the line corresponds to a labelling signal.



Fiber data in GTF format visualization under IGV

**An Example (Quick start):**

java -jar GenerateGTF\_ByAllDataRefining\_Reformat.jar

-I /Volumes/WWT/Reformat/TXT/1802/

-O /Users/wwang/Desktop/ORM/

-SampleName 1802.0a.txt,1802.0b.txt

-Contain T

Notice: The optional parameter is shown in red.

1. GetFiberCoordinate\_ByTXT.jar

The input data needs .txt file(s) got by AllRawDataRefining\_1R2G.jar or AllRawDataRefining\_1G2R.jar. It is used to generate fiber’s bed files.

Parameters:

-I: The parent path of the input txt file(s)

-O: The path of output file(s)

-SampleName: the name of all txt file, multi files will be separated by “,” and the name of txt file can’t contain character “\_”, since it has been used as delimiter in script.

**An Example (Quick start):**

java -jar GetFiberCoordinate\_ByTXT.jar

-I /Volumes/WWT/Final-Version/1807\_1802\_1905\_1708\_Merge/TXT/AlldataRefining/0min/

-O /Users/wwang/Desktop/ORM/GetFiberCoordinate\_ByTXT/Test

-SampleName 1905.FC0.txt,1905.FC1.txt

1. GetRedflagNumberInSlidingWindow\_ByAllDataRefining.jar

The input data needs .txt file(s) got by AllRawDataRefining\_1R2G.jar or AllRawDataRefining\_1G2R.jar. It is used to calculate the signal counts within sliding windows.

Parameters:

-I: The parent path of the input txt file(s)

-O: The path of output file(s)

-SampleName: the name of all txt file, multi files will be separated by “,” and the name of txt file can’t contain character “\_”, since it has been used as delimiter in script.

-REF: The input REF file for the reference chromosome length, unit nt, see below for an example (can find for example at UCSC genome browser):

chr1 249250621

chr2 243199373

chr3 198022430

…

Please pay attention, here the chromosome names must be the same as the chromosome names show up in .txt files (case sensitive, i.e. Chr and chr is different). If using chr23 and chr24 to represent chrX and chrY, respectively, in .txt file, the corresponding chromosome names in this REF file should be chr 23 and chr 24 instead of chrX and chrY.

-WS: window size, Unit kb

-SS: SlidingStep size, Unit kb

**An Example (Quick start):**

java -jar GetRedflagNumberInSlidingWindow\_ByAllDataRefining.jar

-REF /Users/wwang/Desktop/enrichment/ChrLength\_hg19.txt

-WS 10

-SS 1

-I /Volumes/WWT/Final-Version/1905/TXT/AllDataRefining/

-SampleName 1905.FC1.txt,1905.FC2.txt

-O /Users/wwang/Desktop/ORM/GetRedflagNumberInSlidingWindow\_ByAllDataRefining/Test

\*calculation of signal numbers within 10kb sliding window with 1kb step.

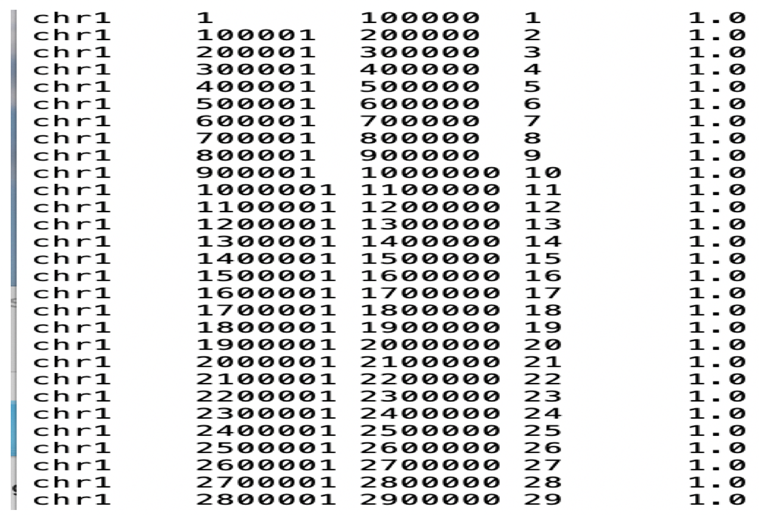
**Output format**

**The 8 columns in the output file corresponding to:**   
Chr Bin\_start Bin\_end Bin\_ID RedsignalNumber FiberNumber NormalizedSignalNumber FiberwithoutSignal  
  
where NormalizedSignalNumber = RedsignalNumber / FiberNumber , which is the signal count after fiber depth normalization.

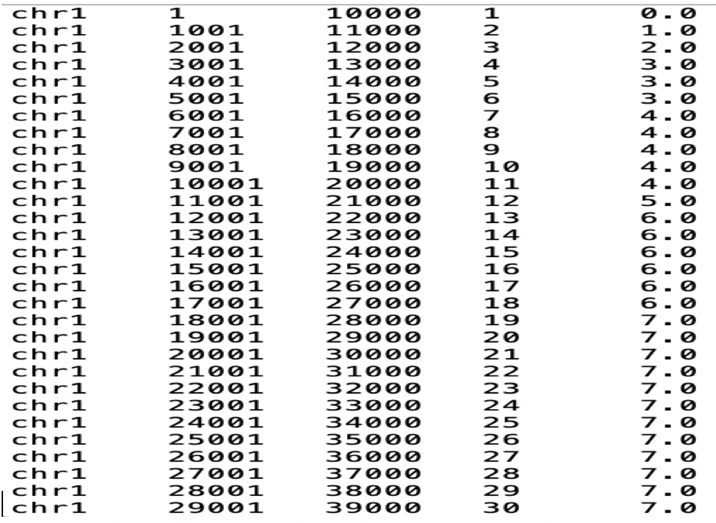
1. GetRedflagNumberInAdjacentWindow\_ByAllDataRefining.jar

It is similar as GetRedflagNumberInSlidingWindow\_ByAllDataRefining.jar but calculates signal number count within continuing non-overlapping windows.

**Output format**   
Non-overlapping adjacent Bins (in case window\_size=100kb)



Sliding Bins (in case window\_size=100kb, SlidingStep=1kb)



**An Example (Quick start):**

java -jar GetRedflagNumberInSlidingWindow\_ByAllDataRefining.jar

-REF /Users/wwang/Desktop/enrichment/ChrLength\_hg19.txt

-WS 10

-I /Volumes/WWT/Final-Version/1905/TXT/AllDataRefining/

-SampleName 1905.FC1.txt,1905.FC2.txt

-O /Users/wwang/Desktop/ORM/GetRedflagNumberInSlidingWindow\_ByAllDataRefining/Test

1. GetNewSegmentation\_AddSoloSignal.jar  
   This jar package is used for clustering neighbored signals based on the distance cutoff values set by user to get the final segments (i.e. ORM tracks in our case). It’s a two-step process: it clusters at first the close signals to get the primary segments then further fusion the close primary segments into the final segments. In order to get the proper cutoff values used in each step for different experiment data, this jar package also calculates the statistics on the distribution of distance between raw signals and the distance between primary segments once the first cutoff for adjacent signals being set. In addition, the users can choose whether including the single signal into the segmentation results (in case they have strong confidence on their data, which are real signals rather than technical noise) by setting -AddSolo options as T. If so, the single signal will become a solo segment with same start and end coordinate in the output file.

Parameters:

-I: The parent path of the input txt file(s)

-O: The path of output file(s)

-SampleName: the name of all txt file, multi files will be separated by “,” and the name of txt file can’t contain character “\_”, since it has been used as delimiter in script.

-GetSignalDistance: if it is “T” the script will generate a document recording the distances between adjacent signals, and the default value is “false”.

-FC: The first cutoff value(s) to cluster the close signals. Multi cutoff values for different files could be separate by “,”. All signals with distance smaller than the corresponding cutoff will be clustered into a primary segment.

-GetSegmentDistance: if it is “T” the script will generate a document recording the distances between adjacent primary segments, and the default value is “false”. And you should put the -FC options prior to -GetSegmentDistance, since the primary segments needed for the analysis depend on the cutoff value set by -FC.

-SC: The second cutoff value(s) to cluster the close segments. Multi cutoff values for different files could be separate by “,”. All primary segments with distance smaller than the corresponding cutoff will be clustered into a final segment, and the script will generate the final bed file(s).

"-AddSolo": If it is “T”, the final result will contain all single signals (each as a solo signal segment) that are not merged into segments in previous steps. The default is “false”;

**Examples (Quick start):**

java -jar GetNewSegmentation\_AddSoloSignal.jar

-GetSignalDistance

-I /Volumes/WWT/Final-Version/1905/TXT/AllDataRefining/

-SampleName 1905.FC1.txt,1905.FC2.txt

-O /Users/wwang/Desktop/ORM/GetNewSegmentation\_AddSoloSignal/Test

java -jar GetNewSegmentation\_AddSoloSignal.jar

-FC 16384,16384

-GetSegmentDistance

-I /Volumes/WWT/Final-Version/1905/TXT/AllDataRefining/

-SampleName 1905.FC1.txt,1905.FC2.txt

-O /Users/wwang/Desktop/ORM/GetNewSegmentation\_AddSoloSignal/Test

java -jar GetNewSegmentation\_AddSoloSignal.jar

-FC 16384,16384

-SC 32768,32768

-I /Volumes/WWT/Final-Version/1905/TXT/AllDataRefining/

-SampleName 1905.FC1.txt,1905.FC2.txt

-O /Users/wwang/Desktop/ORM/GetNewSegmentation\_AddSoloSignal/Test

(-AddSolo T)

1. GMM.R

This is an R package used for drawing gaussian mixture model distributions. In order to better set the cutoff values to cluster close signals and primary segments, user can do the gaussian mixture model classification. Most of time we classify into 3 distribution (i.e. corresponding to signals from the same event, signals from adjacent event, and noise, respectively) and choose the tail of first distribution as the cutoff value. The data of distance between signals or distance between primary segments come from jar package GetNewSegmentation\_AddSoloSignal.

The user needs to set the Input\_ParentPath, Output\_ParentPath, Names of all samples (the same as indicated in other packages) and the X axis’ range (i.e. limit for the X axis) for the plot.

1. Add\_FDI\_ToSegment.jar

-FDI\_N: the segments with negative FDI values and between 0 and this cutoff are filtered out.

-FDI\_P: the segments with positive FDI values and between 0 and this cutoff are filtered out.

-Signal\_N: The cutoff value for filtering the segments with negative FDI containing signals smaller than the cutoff

-Signal\_P: The cutoff value for filtering the segments with positive FDI containing signals smaller than the cutoff

-I-T: The parent path of the input txt file(s)

-I-B: The parent path of the input bed file(s)

-SampleName: the name of all input file, multi files will be separated by “,” and the name of txt file can’t contain character “\_”, since it has been used as delimiter in script. Please pay attention the .txt and .bed files should show up in pair. The .bed file is generated by script GetNewSegmentation\_AddSoloSignal. And the name of the pair should be same except suffix, for example Sample1.txt and Sample1.bed

-O: The path of output file(s)

**An Example (Quick start):**

Java -jar Add\_FDI\_ToSegment.jar

-FDI\_N -0.6

-FDI\_P 0.8

-Signal\_N 3

-Signal\_P 3

-I-T /Volumes/WWT/Final-Version/1905/TXT/AllDataRefining/

-I-B /Volumes/WWT/Final-Version/1905/Bed/

-SampleName 1905.FC1,1905.FC2

-O /Users/wwang/Desktop/ORM/Add\_FDI\_ToSegment/Test/

**Functions specific for DNA replication data**

1. GetS50Timing.jar

Replication timing data can be added into the analysis. For example, in our study, we include the replication timing obtained by Repli-Seq, defined as S50, the fraction of the S phase (0<S50<1) at which 50% of DNA is replicated in a defined region, which is computed by a linear interpolation of enrichment values of Repli-Seq signals obtained within six S phase compartments. It can reflect the temporal order of replication along the genome. To run this package, you need to provide S50 timing along the genome. It is 23 one column files which recorded all the S50 value in 1kb sliding step for 100kb window along the genome.

Parameters:

-I: the parent path of the bed file(s) that you want to add S50 values. Please pay attention the input bed file(s) should be sort by chromosome order, and the chromosome names need to match between the bed file(s) and the S50 file.

-O: the parent path of the output file(s)

-S50: the parent path of the document containing the one column S50 values along the genome

-SampleName: the name of all input file, multi files will be separated by “,” and the name of bed file can’t contain character “\_”, since it has been used as delimiter in script.

**An Example (Quick start):**

Java -jar Add\_FDI\_ToSegment.jar   
 -I /Volumes/WWT/Final-Version/1905/Bed/sort   
 -O /Users/wwang/Desktop/ORM/GetS50Timing/Test   
 -S50 /Users/wwang/Desktop/cluster/S50\_1kbSlide\_100kbWindow/   
 -SampleName 1905.FC0.bed,1905.FC1.bed

1. Add\_DeltaRFD\_ToSegment.jar

In DNA replication field, the RFD (Replication Fork Directionality) curve obtained by OK‑Seq method can be used to call initial zones. It is calculated by the numbers of Okazaki fragments corresponding to leftward (L) and rightward (R) replication forks, RFD=(R-L)/(R+L). The RFD values that we used are computed in 1 kb adjacent non-overlapping windows. When crossing replication origins, RFD curve shows a sharp increasing trend, so the difference of RFD values in the end and start position of a replication origin, called it Delta\_RFD values, are positive. The larger the Delta\_RFD values is the higher firing efficiency of corresponding replication origin. See ([REF](https://www.nature.com/articles/ncomms10208#Sec14)) for more detail.  
  
-I: the parent path of the bed file(s) that you want to add Delta\_RFD values. Please pay attention the input bed file(s) should be sort by chromosome order, and the chromosome names need to match between the bed file(s) and the RFD file.

-O: the parent path of the output file(s)

-RFD: the parent path of the document containing all RFD values in 1kb bin along the genome (in bedgraph format)

-SampleName: the name of all input file, multi files will be separated by “,” and the name of bed file can’t contain character “\_”, since it has been used as delimiter in script.

**An Example (Quick start):**

Java -jar Add\_DeltaRFD\_ToSegment.jar

-SampleName 1905.FC0.bed,1905.FC1.bed

-O /Users/wwang/Desktop/ORM/Add\_DeltaRFD\_ToSegment/Test

-I /Volumes/WWT/Final-Version/1905/Bed/sort

-RFD /Users/wwang/Desktop/Final-Version/ForkDirection/AddFDItoBed/RFD\_InWindow/output/1kb/NewRFD\_1kb\_sort.bedgraph

1. Calculate\_FDI\_RFD

Similar to RFD, the FDI\_RFD is also calculated by formula (R-L)/(R+L), but L and R correspond to the numbers of ORM segments with positive and negative FDI values, respectively, overlapping with each 1kb window. Firstly, the segment bed file(s) should be treated by the package Add\_FDI\_ToSegment.jar, then we can map these regions into 1kb adjacent bins along the genome and calculate the corresponding FDI\_RFD values.

-REF: The input REF file for the reference chromosome length, unit nt, see below for an example (can find for example at UCSC genome browser):

chr1 249250621

chr2 243199373

chr3 198022430

…

Please pay attention, here the chromosome names must be the same as the chromosome names show up in .txt files (case sensitive, i.e. Chr and chr is different). If using chr23 and chr24 to represent chrX and chrY, respectively, in .txt file, the corresponding chromosome names in this REF file should be chr23 and chr 24 instead of chrX and chrY.

-WS: window size, unit kb

-I: The parent path for multi bed files with FDI values or a single bed file with FDI values

-O: The parent path for multi results with FDI\_RFD or a single bed file with FDI\_RFD

-Colum\_FDI: The Colum number containing the FDI values within the input file(s)

-SignalColumn: The Colum number of input file’s signal number. If the bed file is generated by jar package GetNewSegmentation\_AddSoloSignal.jar, there should be one column is signal number. If the bed file contains this column, user can set the cutoff for filtering segments with few signals to reduce the false positive. The suggested cutoff is 3. The default value is 2, if not set.

-FDI\_N\_Cutoff: The cutoff value for filtering the segments with negative FDI. The default will be zero if not set.

-FDI\_P\_Cutoff: The cutoff value for filtering the segmentation with positive FDI. The default will be zero if not set.

-Signal\_N: The cutoff value for filtering the segments with negative FDI containing signals lower than the cutoff. The default will be zero if not set.

-Signal\_P: The cutoff value for filtering the segments with positive FDI containing signals lower than the cutoff. The default will be zero if not set.

**An Example (Quick start):**

Single bed input with FDI cutoff setting

Java -jar -REF  /Users/wwang/Desktop/enrichment/ChrLength\_hg19.txt

-WS 1

-I /Users/wwang/Desktop/ORM/FDI\_RFD/Test/All0min.bed

-O /Users/wwang/Desktop/ORM/FDI\_RFD/Test/Output.bed

-Colum\_FDI 7

-SignalColumn 5

-SignalCutoff 2

-FDI\_P\_Cutoff 1

-FDI\_N\_Cutoff -1

Multi bed input with default FDI cutoff

Java -jar -REF  /Users/wwang/Desktop/enrichment/ChrLength\_hg19.txt

-WS 1

-I /Users/wwang/Desktop/ORM/FDI\_RFD/Test/

-O /Users/wwang/Desktop/ORM/FDI\_RFD/Test/Output.bed

-Colum\_FDI 7

-SignalColumn 5

-SignalCutoff 2

-SampleName 1905.FC0.bed,1905.FC1.bed

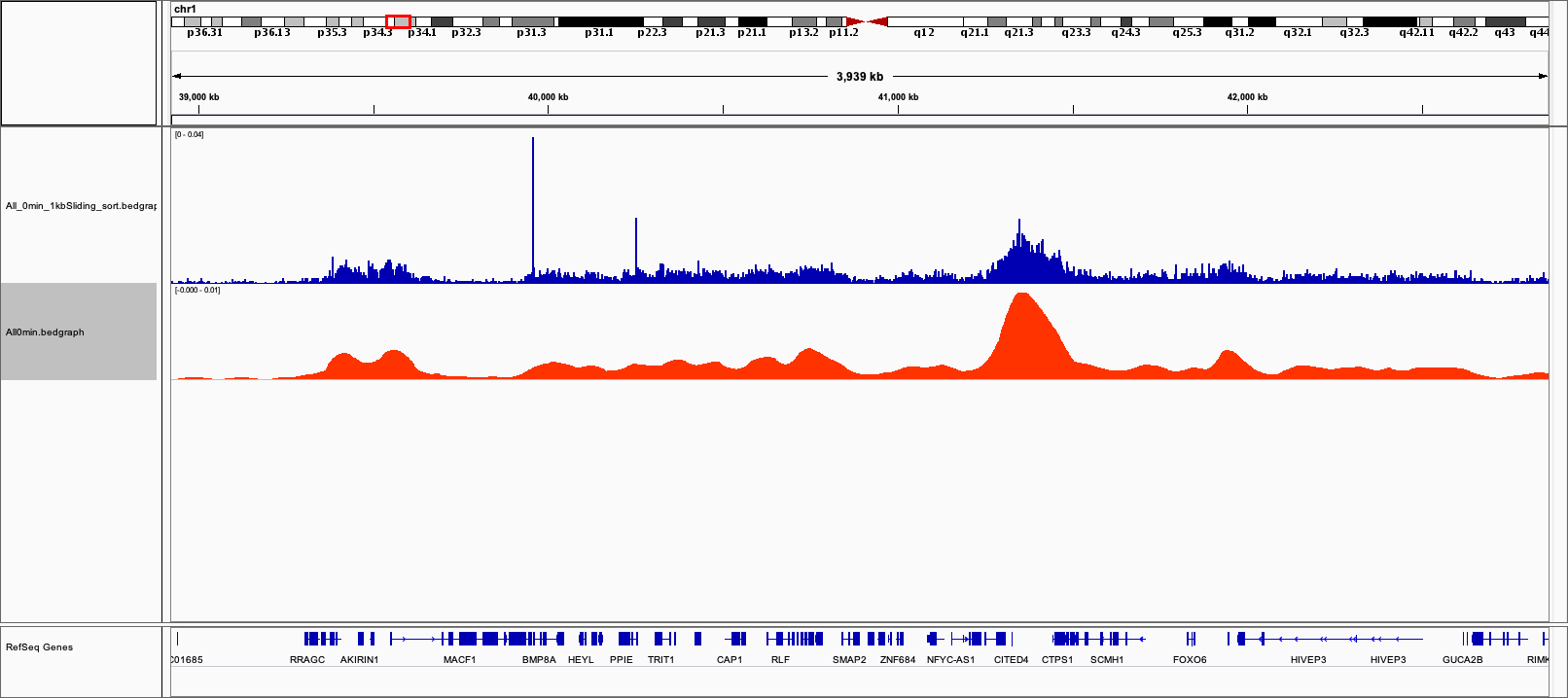
1. LOESS.R  
    The LOESS.R is used to fit the signal fire efficiency in adjacent bins into continuous curve. The input file should have at least 4 columns. The chromosome, start, end and fire efficiency (or other value to smooth). The function Fitcurve() has 4 parameters. The input data, output bin size, transition region length (to eliminate the gap between two adjacent bins) and the signal column for smoothing.

Parameters

1. Input: The data frame of your input file path
2. The length of output bin: Please pay attention the output bin length should be the integer multiple of input bin length, at least same as the input bin size, unit kb.
3. Because the LOESS fitting is impossible to do along the whole chromosome. It will be very time consuming. So, we set a buffer transition area equally divided across 2 adjacent output bins. And just do the LOESS fitting in each output bin and extending half transition area inside the adjacent output bin range, which means within the transition area, all signals are fitted by twice. the final smoothed values are the averages of the smoothed values from two adjacent windows, weighted by their distance to the adjacent window.

**An Example (Quick start):**

Input <- read.table(xxx/Your files’ path)   
Fit <- FitCurve(Input,20,10,4)  
## Annotation: The output bin size is 20kb, transition area length is 10kb, the fire efficiency needs to LOESS smooth is in 4th column



**Comparison between LOESS output signals and raw signals**

1. Multi\_peakcalling.R

After LOESS smoothing fitting by LOESS.R, we can do peak calling for the smoothed fitting signals. There are 2 functions.

Function1: GetInitialZone\_byBin()

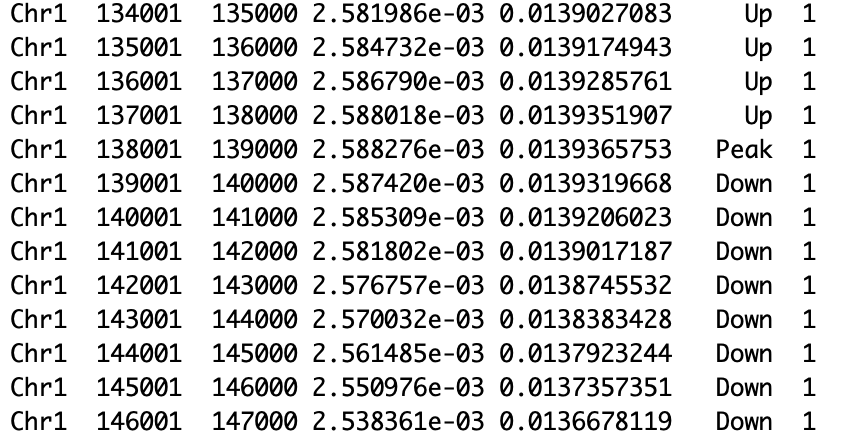
It just needs the LOESS fitting result as input or other continuous fitting signals

An Example (Quick start):

Input <- read.table(xxx/Your LOESS fitting files’ path)   
Peak <- GetInitialZone\_byBin(Input)

**Output format**

**The output file containing 7 columns corresponding to**  
Chr Bin\_start Bin\_end Fitting\_Signal\_value Signal\_Percentage Trend\_Label InitialZone\_Sign



At first, the positions of peaks and the valleys are defined as the local maximum and local minimum, respectively, on the continuous normalized ORM signal density profile. And an initiation zone region is defined from a peak (labeled as Peak at the 6th column) to the closest valleys (labelled as Valley at the 6th column) on both sides. All the bins within such range will be marked as 1 for InitialZone\_Sign column. Then, based on the value of the normalized ORM signal density belong to each bin, if it is bigger or smaller than the adjacent bins, each bin can be labelled as Up or Down, respectively, at the 6th column. Finally, we will calculate the sum of smoothing signal densities in the 4th column within the entire initial zone, and calculate the percentage of each bin’s signal to this sum value reported as signal percentage at the 5th column, which are then used to narrow down to get a more precise definition of an initial zone. Here in our study, based on our investigation, the size of each initiation zones was defined by the smallest window that containing at least 40% of ORM signals corresponding each peak (see our manuscript for more detailed information).

Function2: GetInitialZone()

This function is used to select the initial zones detected in several biological replicates from the outputs obtained by GetInitialZone\_byBin(). It needs 3 parameters, the outputs of GetInitialZone\_byBin(), the vector recoding for each bin in which biological replicate(s) an initiation zone being identified (based on the 7th column), and the label vector for each biological replicates.An Example (Quick start):  
  
S1708 <- read.table("xxx/Your GetInitialZone\_byBin() output")

S1802 <- read.table("xxx/Your GetInitialZone\_byBin() output")

S1807 <- read.table("xxx/Your GetInitialZone\_byBin() output")

S1905 <- read.table("xxx/Your GetInitialZone\_byBin() output ")

IZ <- list(S1708,S1802,S1807,S1905)

Sign <- c("a","b","c","d")

SampleNumber <- rep(0,nrow(IZ[[1]]))

for(i in 1:4)

{

Tmp <- IZ[[i]]

Tmp[,8] <- "0"

Tmp[which(IZ[[i]]$V7 > 0),8] <- Sign[i]

SampleNumber <- paste(SampleNumber,Tmp$V8,sep = "")

}

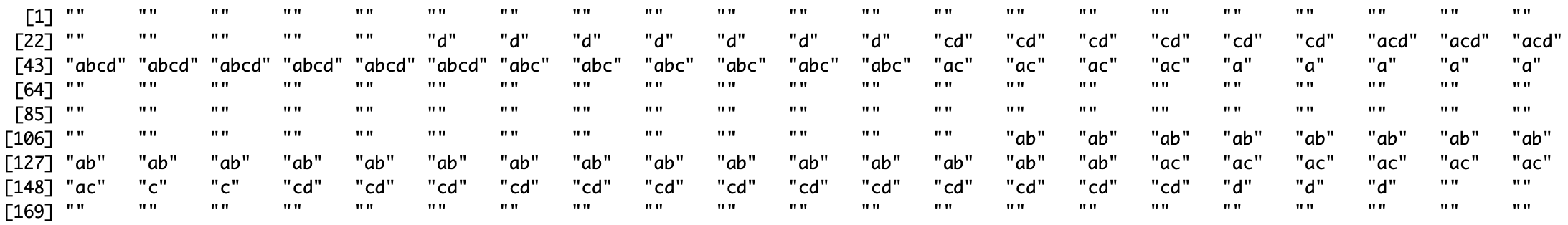
library(stringr)

SN <- str\_replace\_all(SampleNumber, "0", "")

All0min <- read.table("/Users/wwang/Desktop/Final-Version/Final\_Initialzone/IZ\_Update/SignalRatio/Sliding/All0min\_SignalRatio.bed")

FinalIZ <- GetInitialZone(All0min,SN, Sign)

Here we used "a","b","c","d" label for 4 replicates as label vector and SN record all the initial zone show up in each bin like below.



The "" means no replicate has initial zone in this bin and if there are any replicate contain the initial zone it will show up corresponding replicate’s label.