



Multiscale Complex Genomics

# Nucleosome Dynamics:

## A set of tools to analyze and visualize MNase-seq data

Diana Buitrago  
21/09/2018

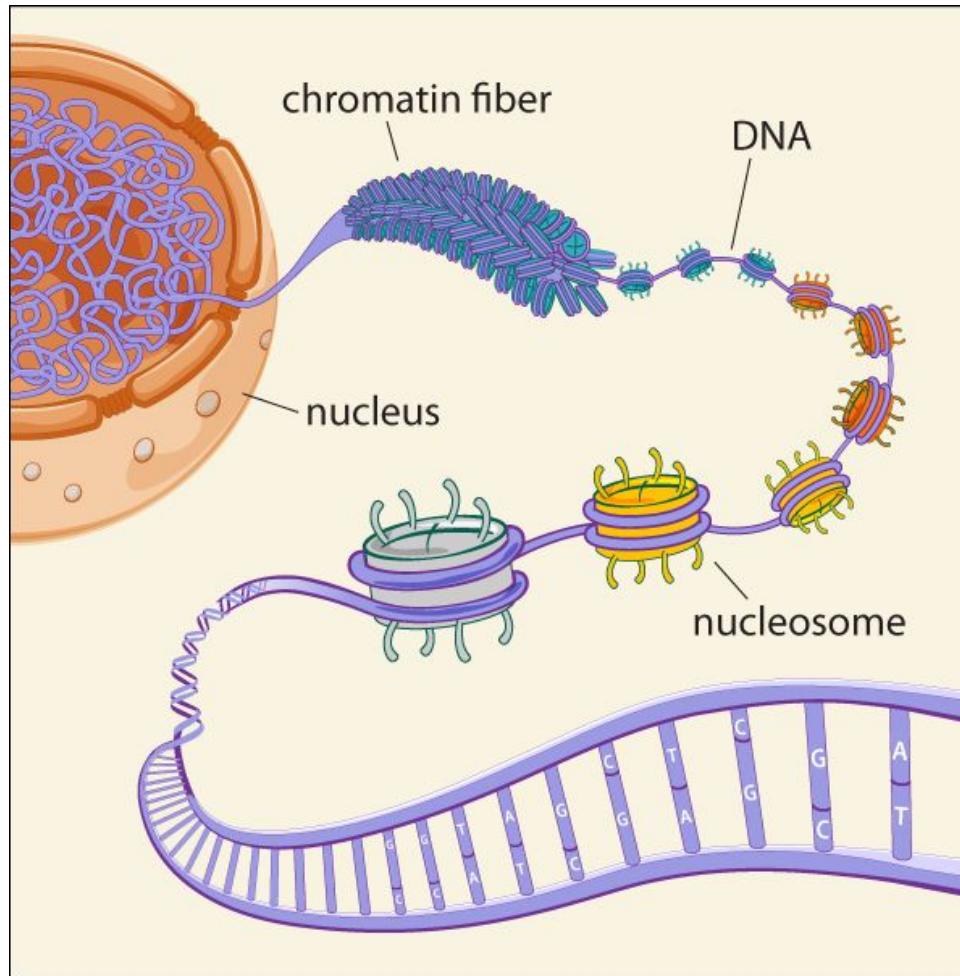


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 676556.



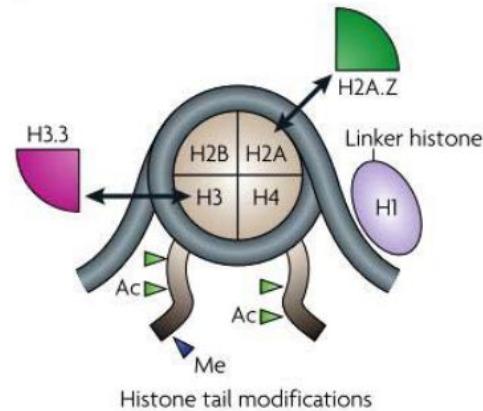


# The basic unit of DNA packaging in eukaryotes

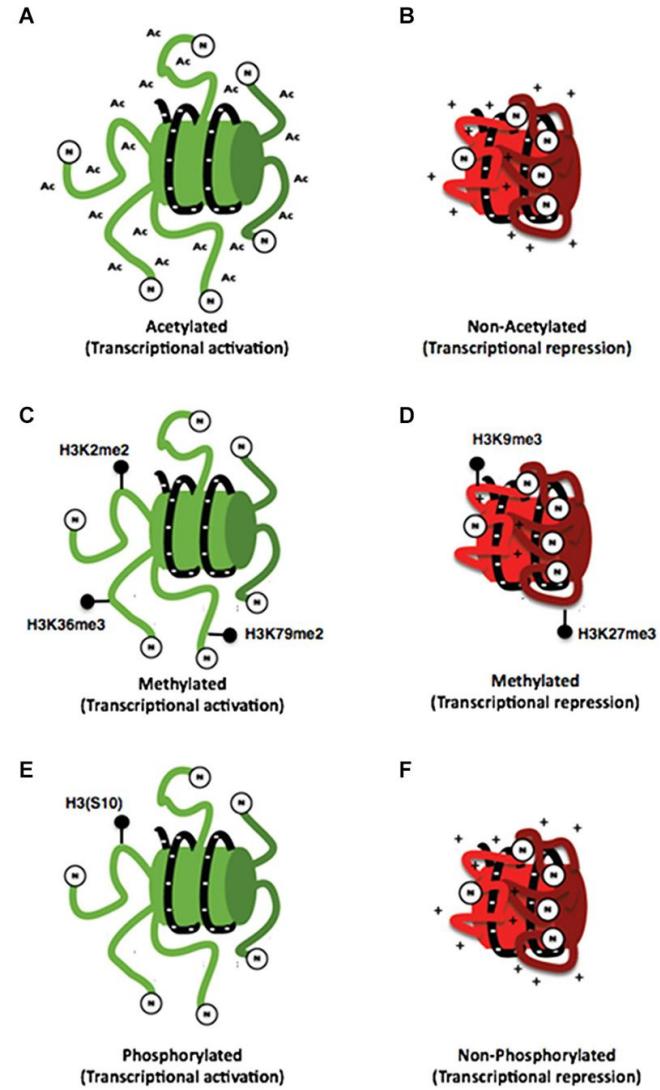




# Nucleosomes can act as epigenetic regulators



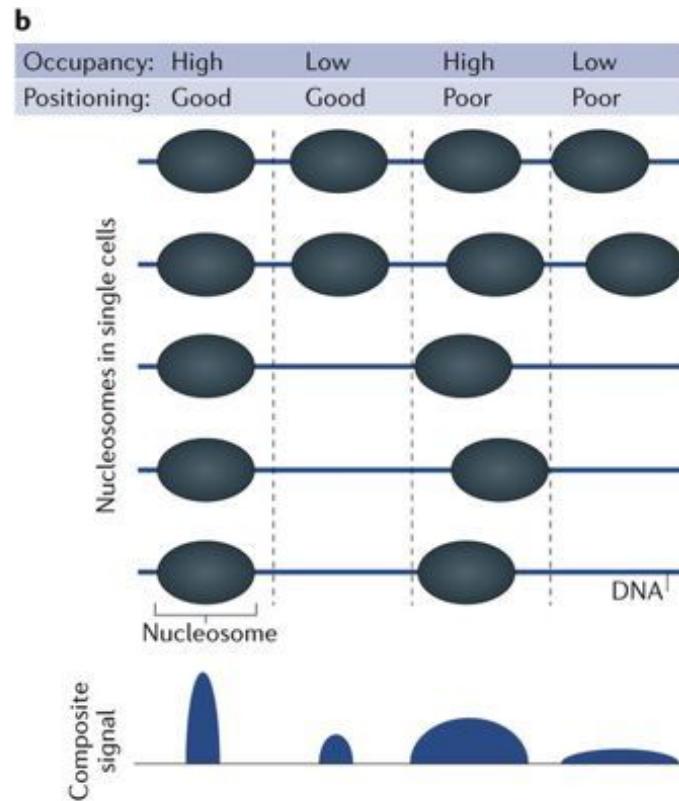
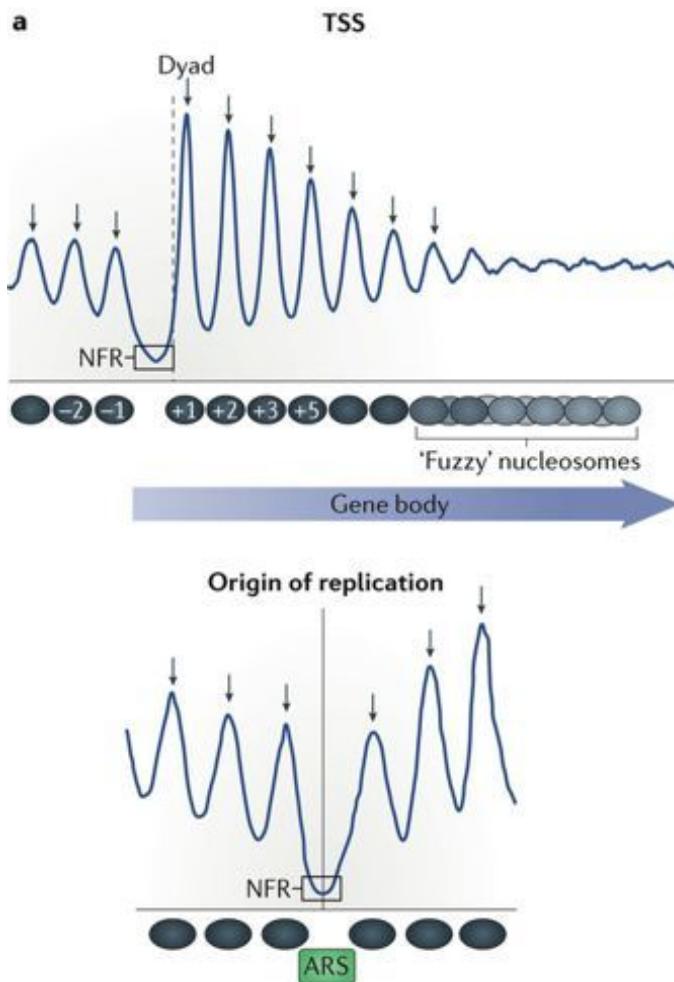
(Jiang and Pugh, 2009)



(Landgrave-Gomez et al. 2015)



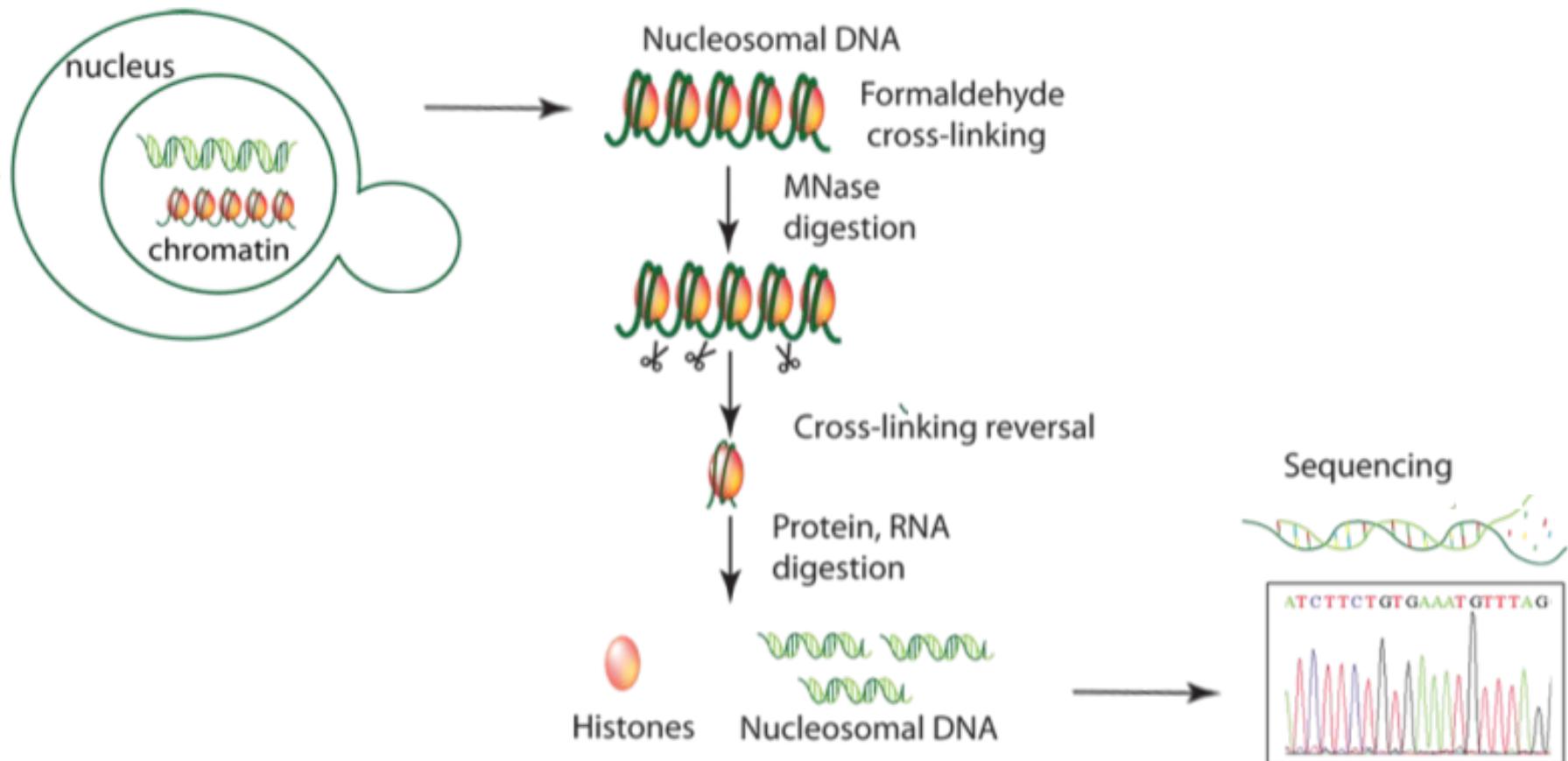
# Nucleosomes are dynamic (sliding, disassemble)



(Lai and Pugh 2017)  
Nature Reviews | Molecular Cell Biology



# MNase-seq is used to determine their position



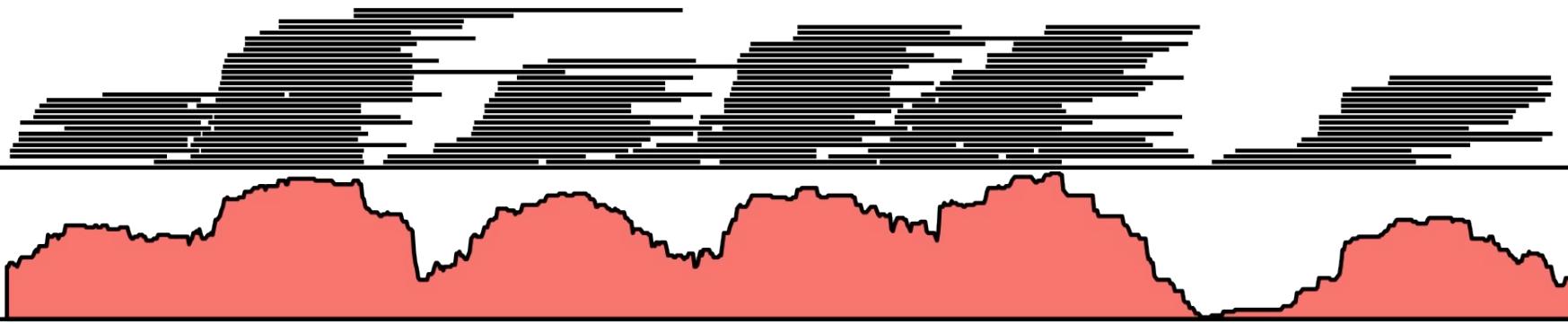


# MNase-seq data processing using nucleR



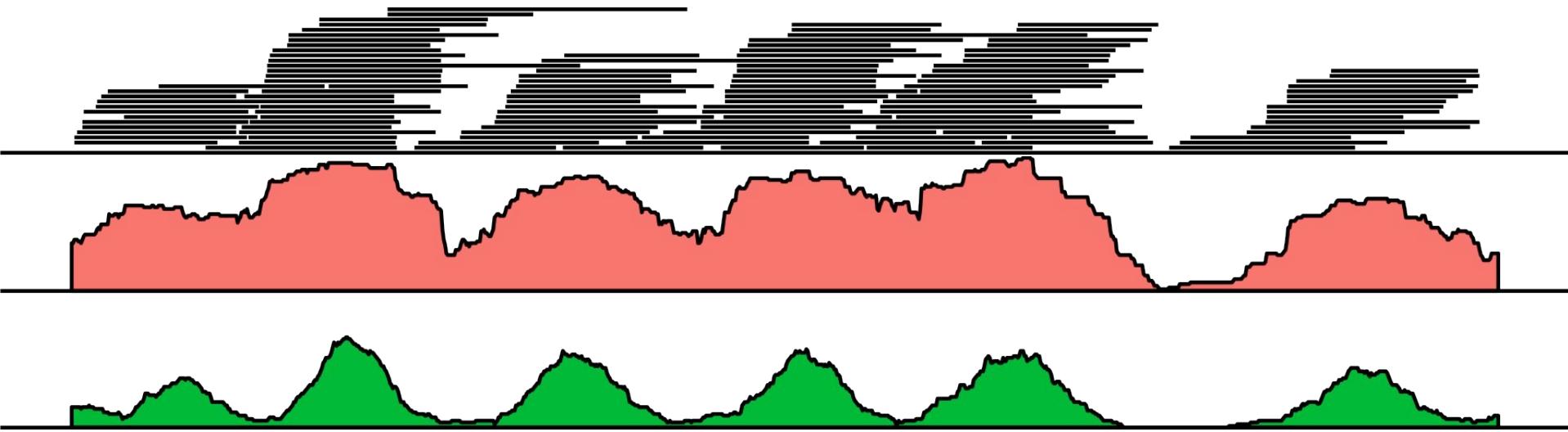


# MNase-seq data processing using nucleR



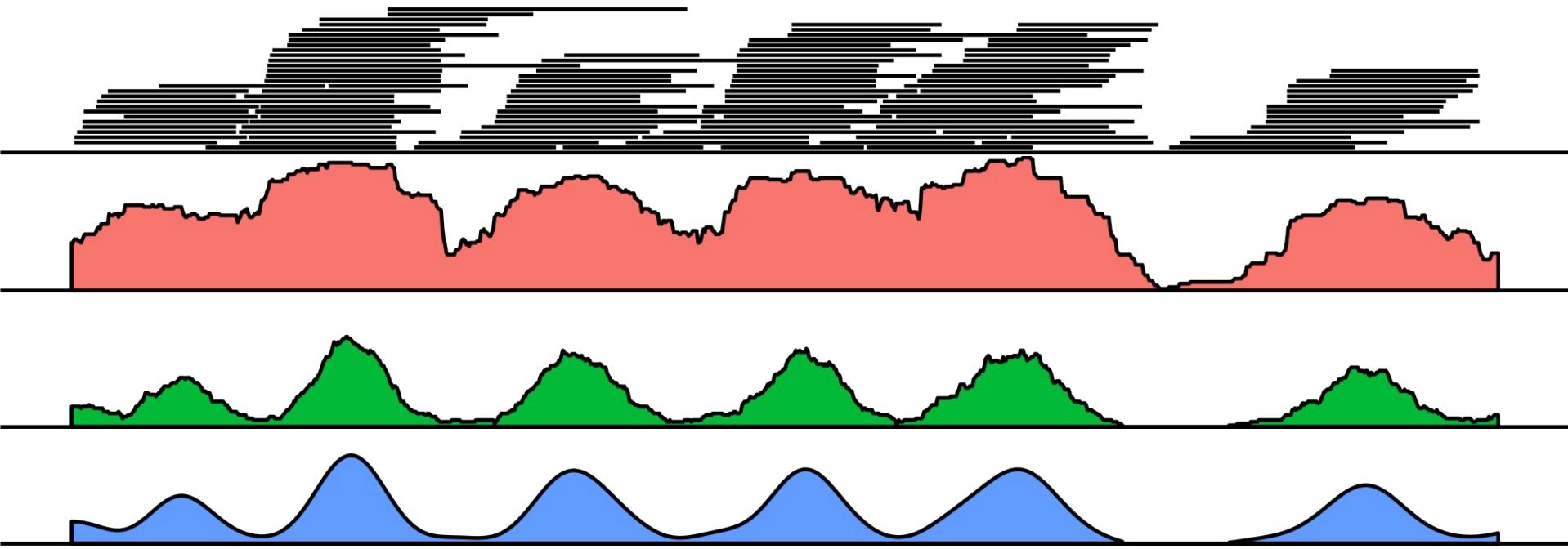


# MNase-seq data processing using nucleR



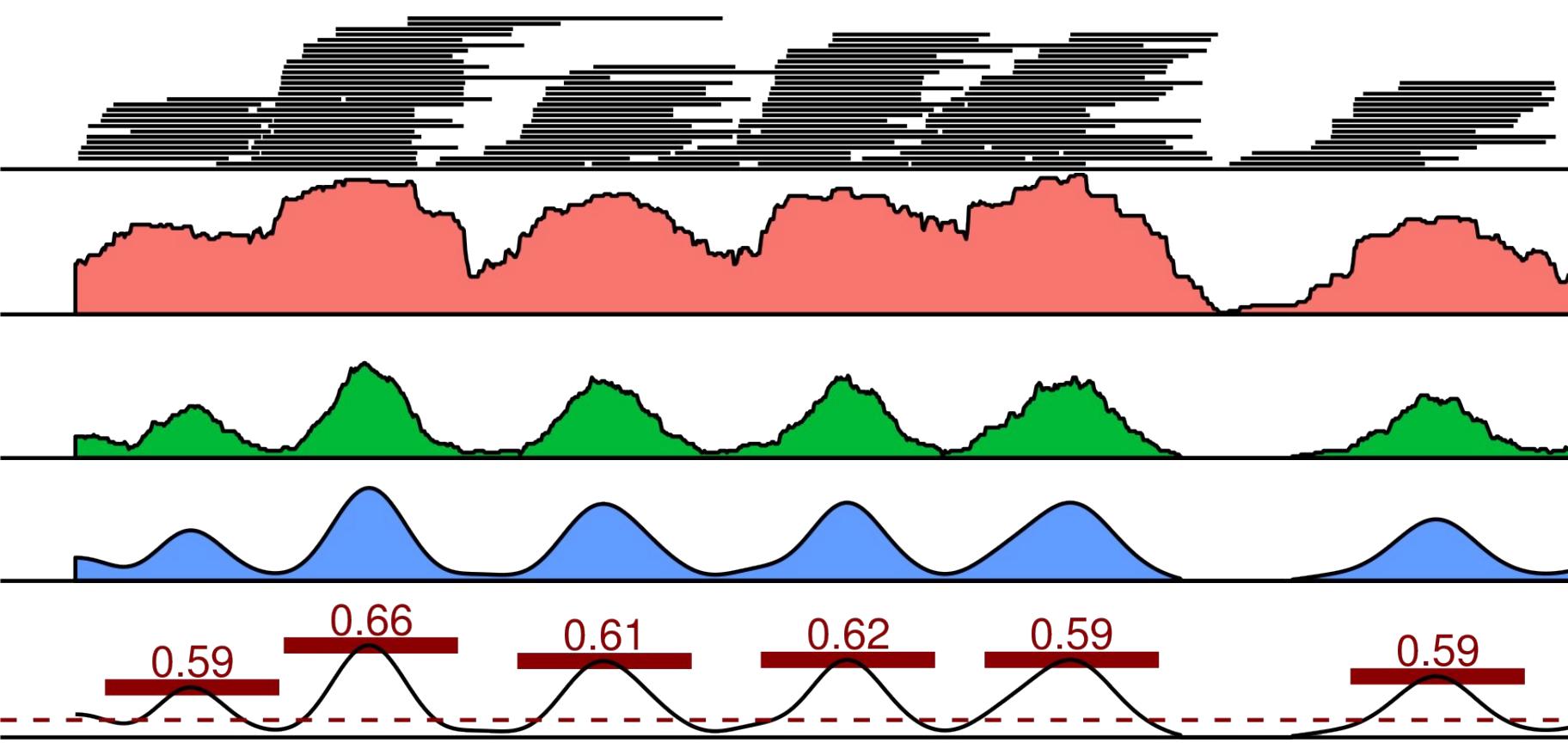


# MNase-seq data processing using nucleR



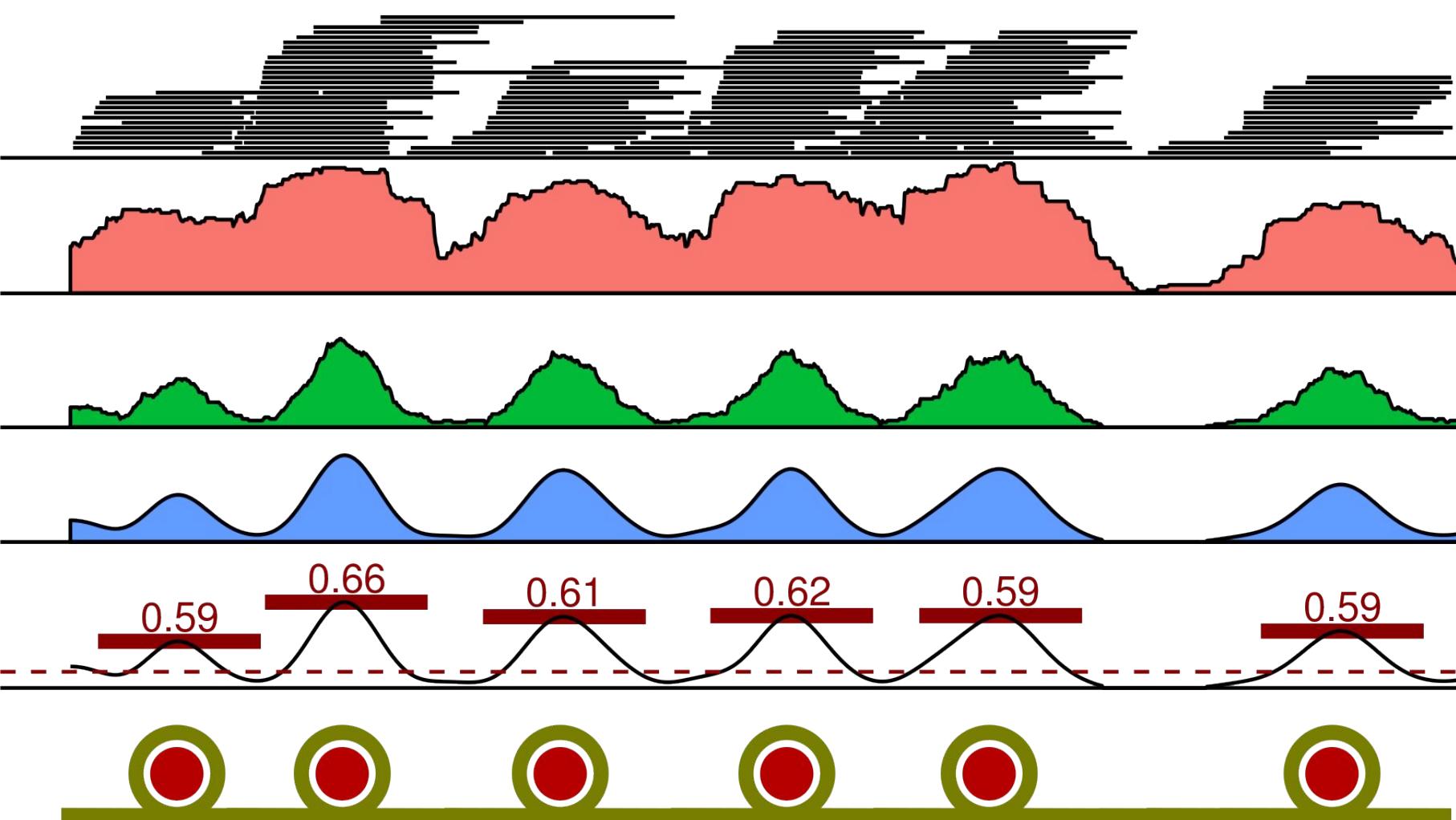


# MNase-seq data processing using nucleR



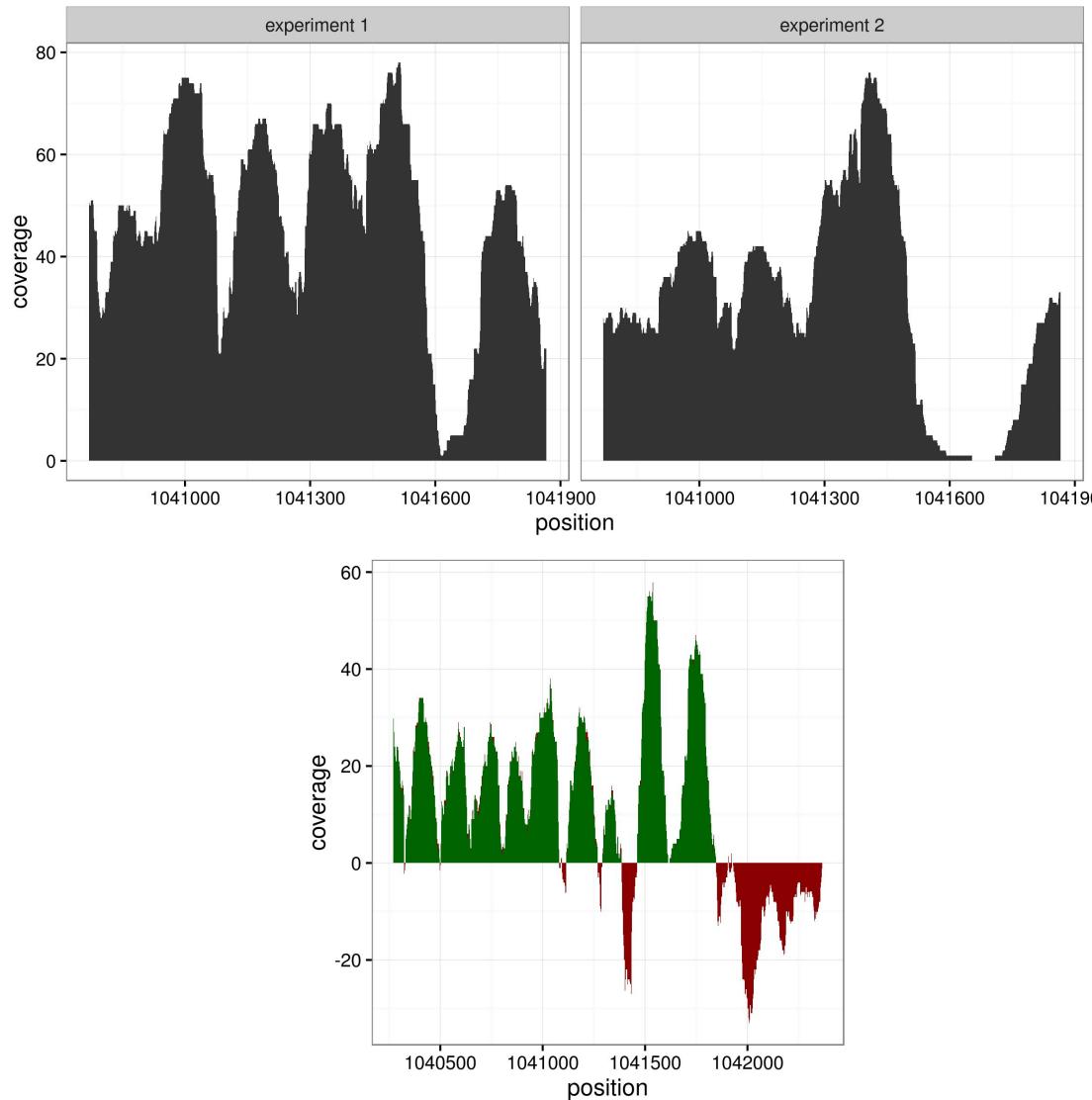


# MNase-seq data processing using nucleR



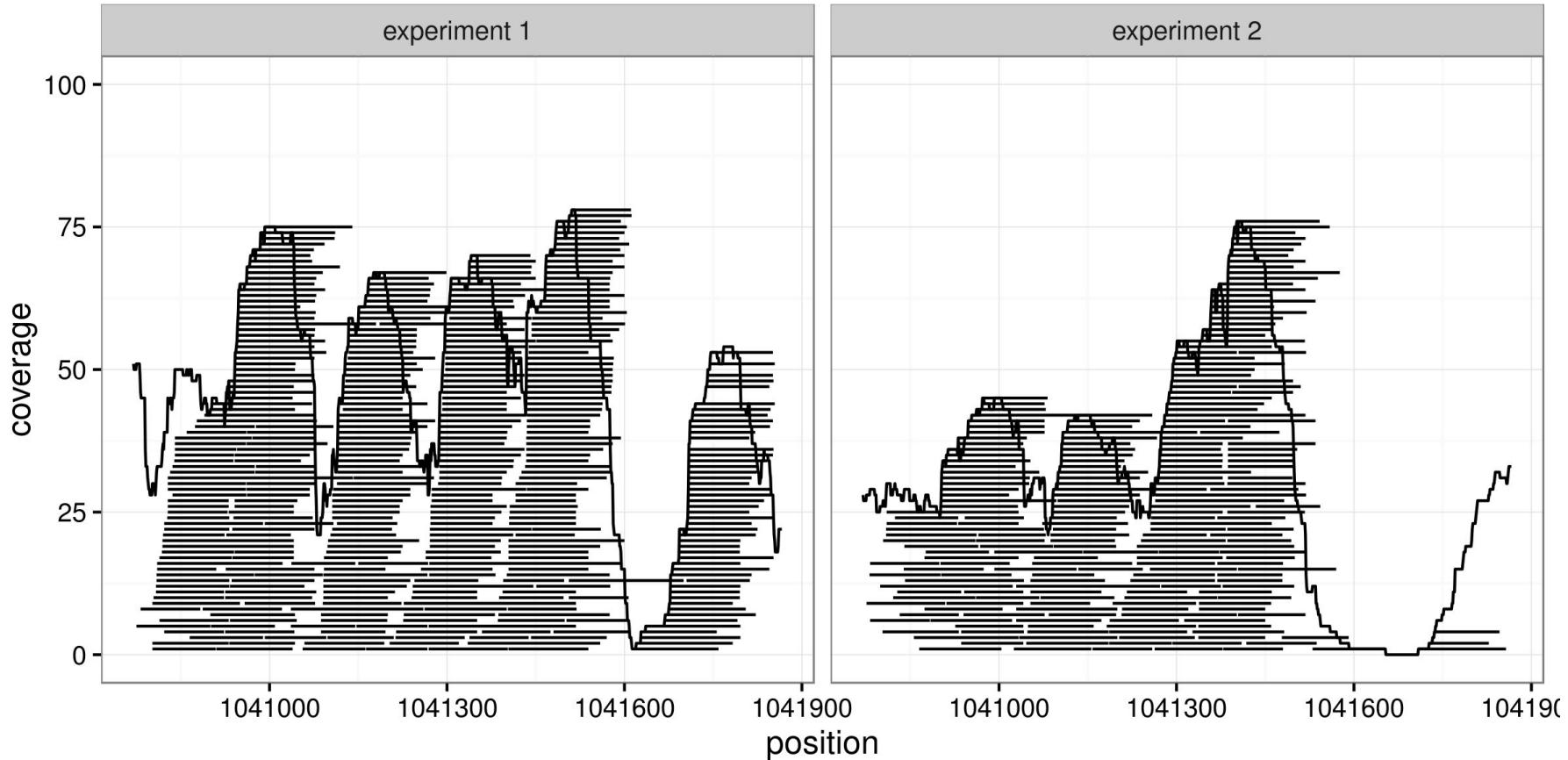


# What about comparing different experiments?



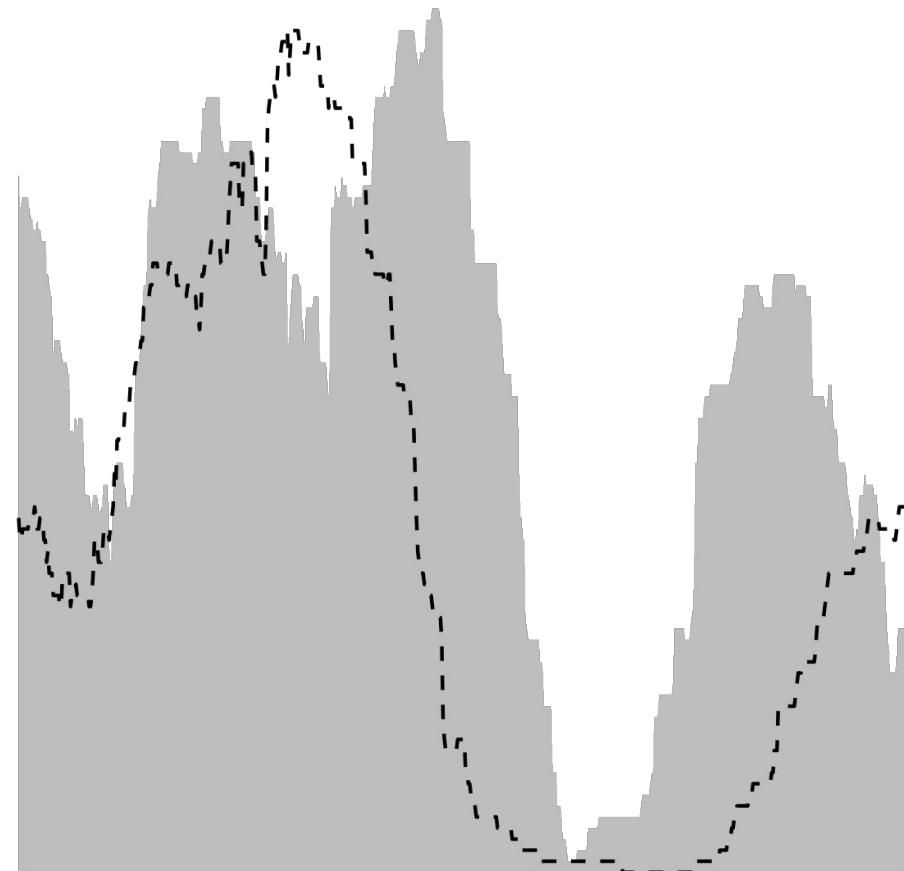


# Nucleosome Dynamics works on a fragment level





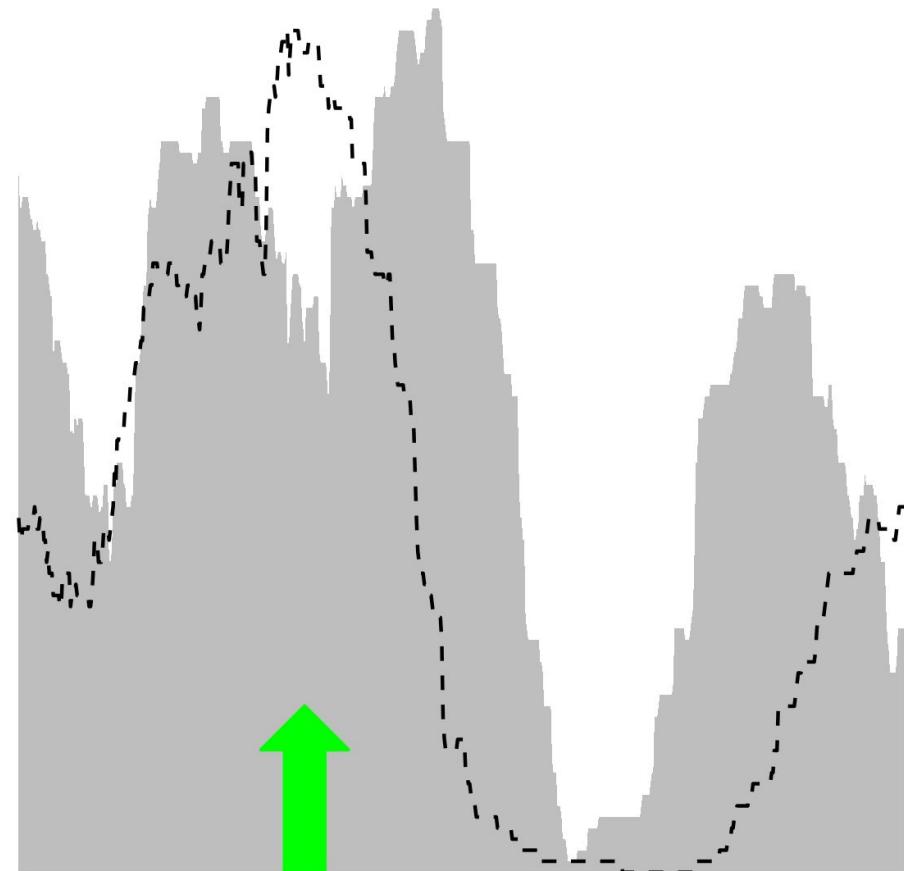
# Nucleosome Dynamics works on a fragment level





# Nucleosome Dynamics works on a fragment level

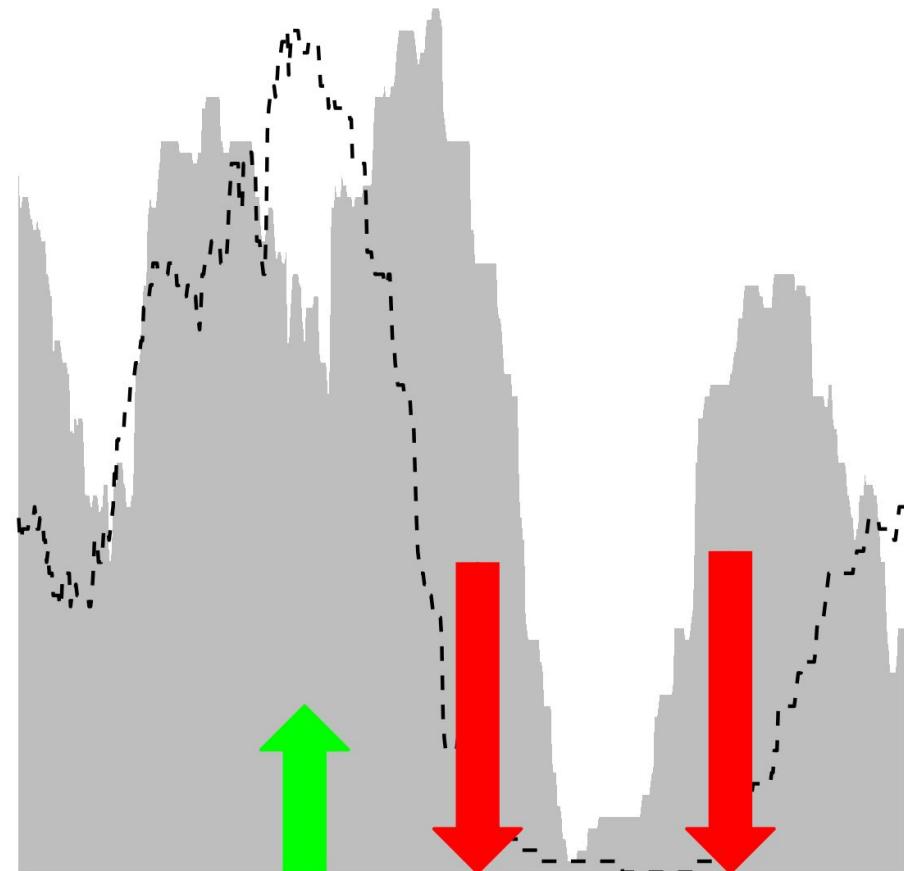
Insertions





# Nucleosome Dynamics works on a fragment level

Insertions  
Deletions



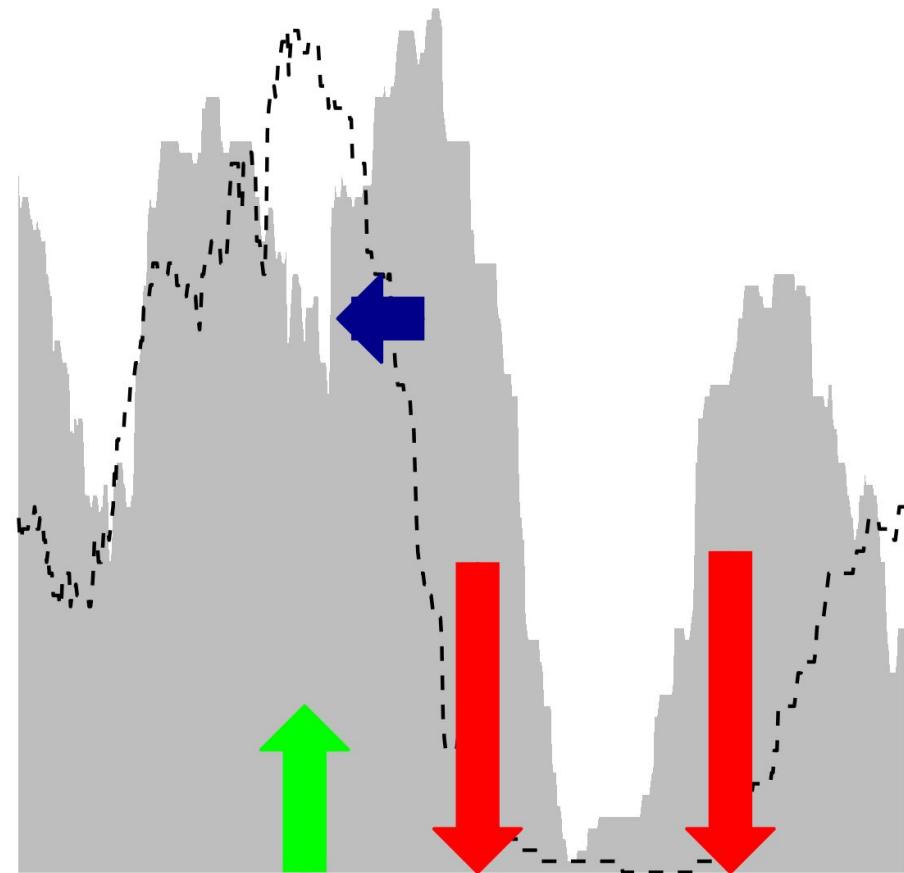


# Nucleosome Dynamics works on a fragment level

Insertions

Deletions

Negative shifts





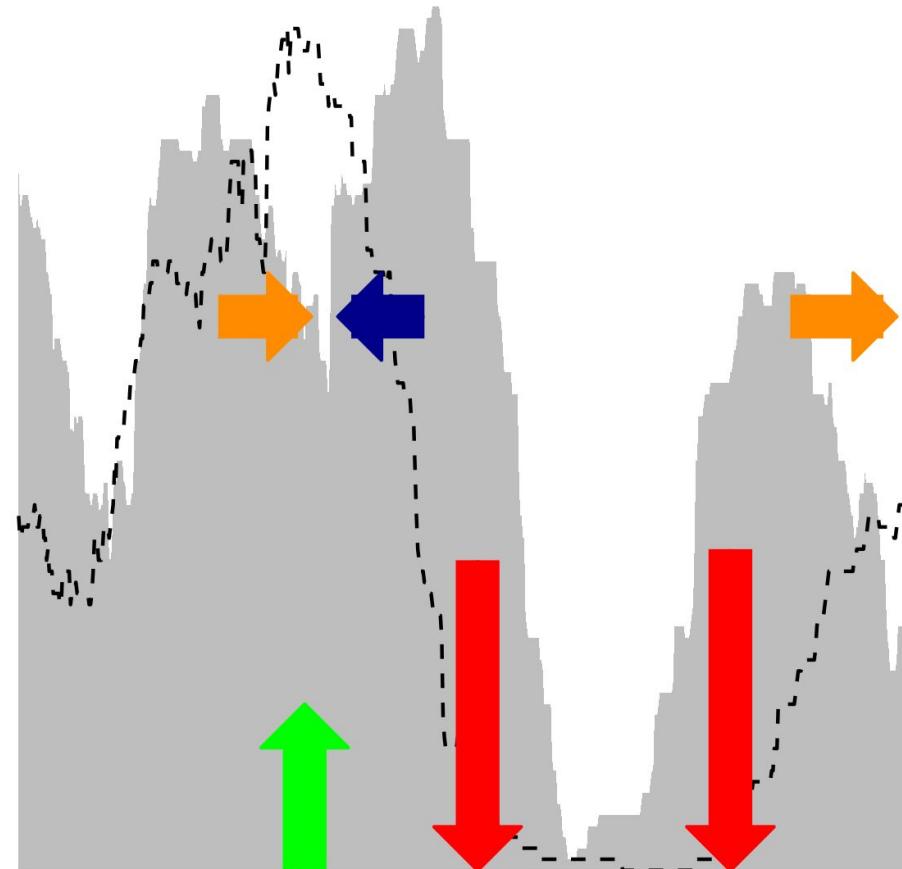
# Nucleosome Dynamics works on a fragment level

Insertions

Deletions

Negative shifts

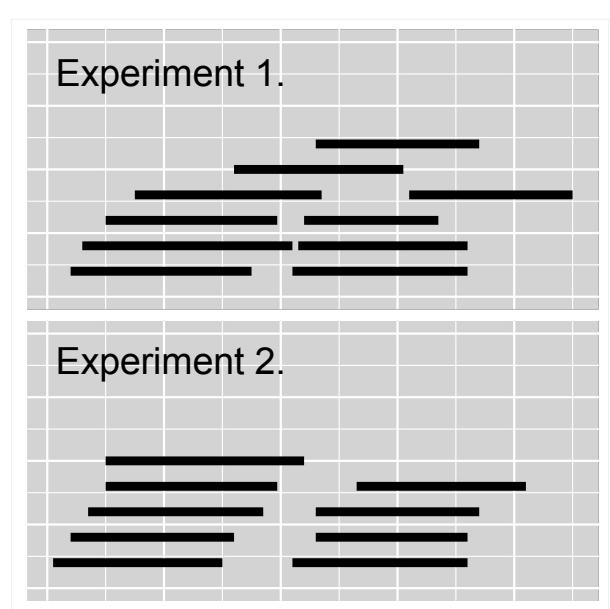
Positive shifts





# Nucleosome Dynamics workflow

**Whole set of reads**





# Nucleosome Dynamics workflow

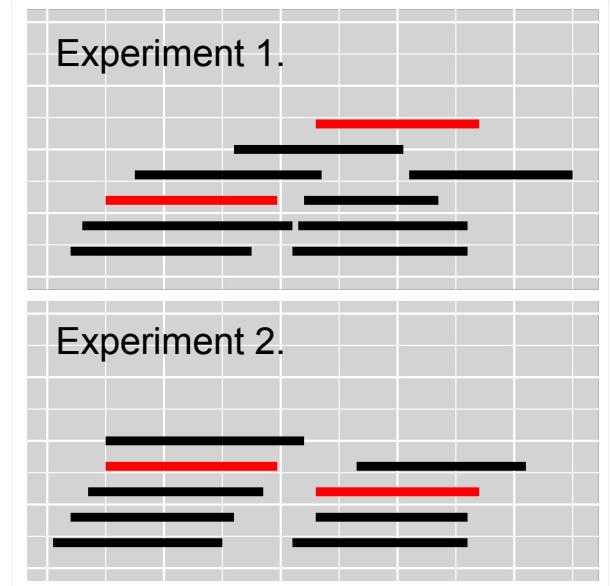
Whole set of reads

Same start and same end in both sets



Experiment 1.

Experiment 2.





# Nucleosome Dynamics workflow

Whole set of reads

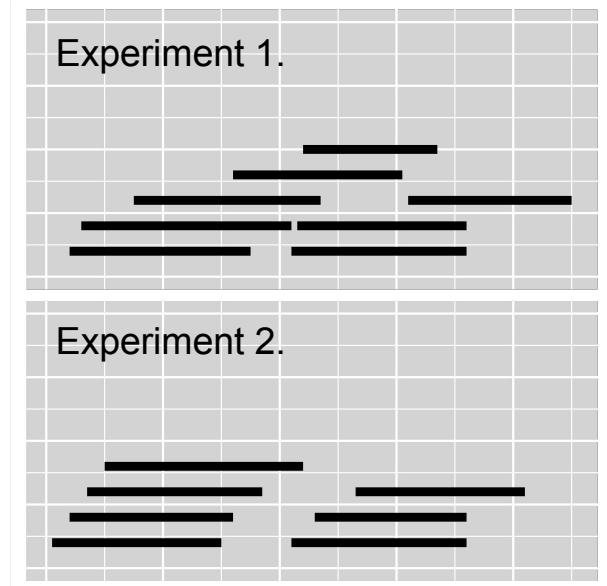


Same start and same end in both sets



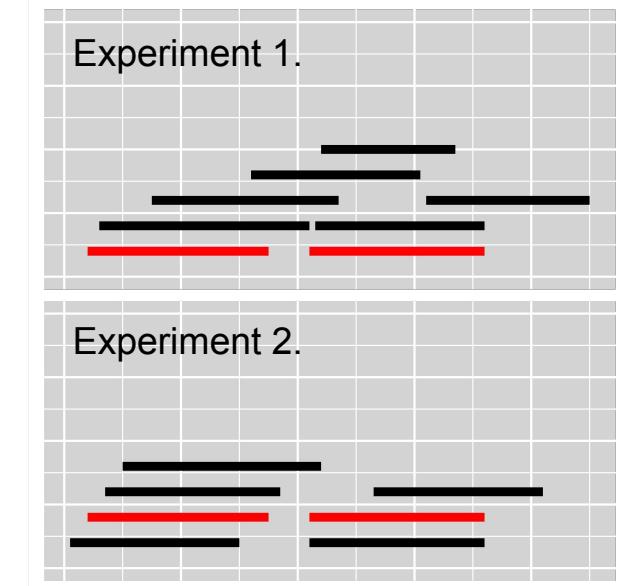
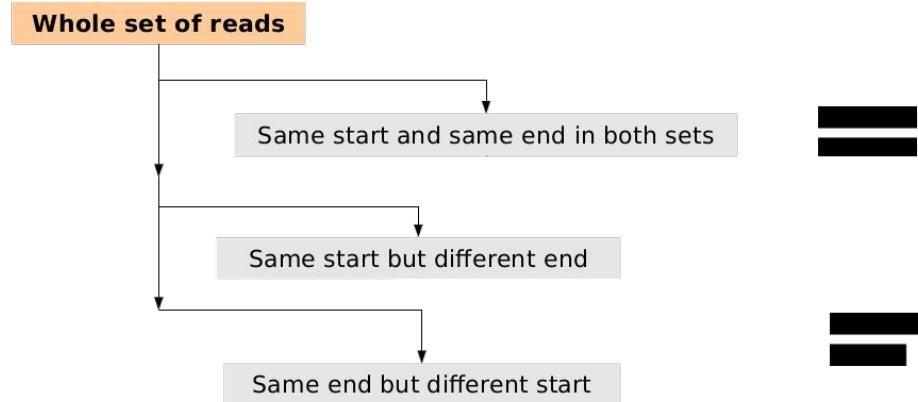
Experiment 1.

Experiment 2.



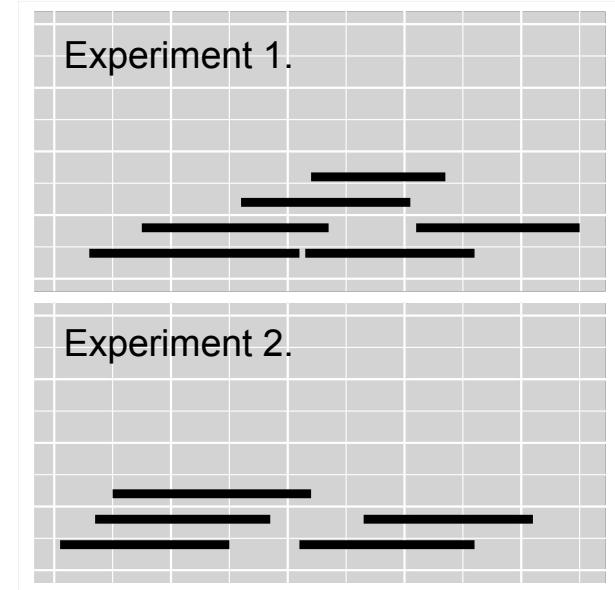
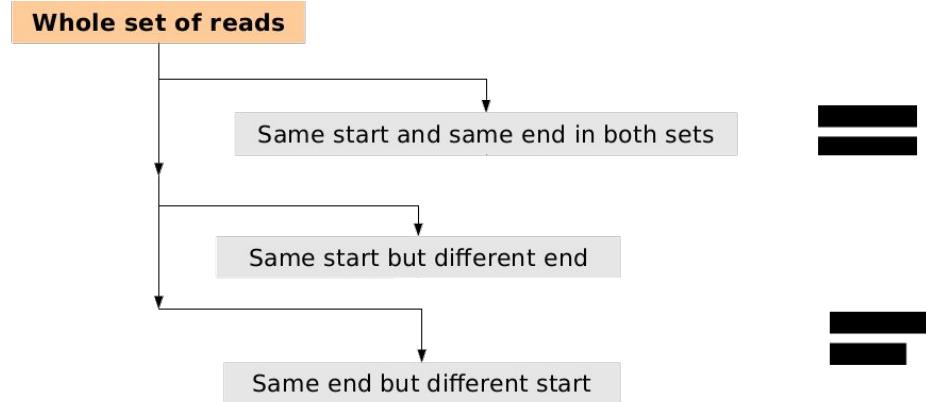


# Nucleosome Dynamics workflow



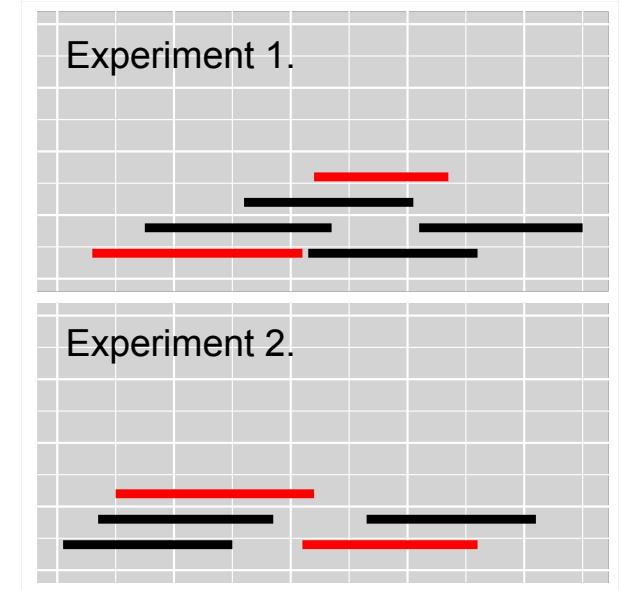
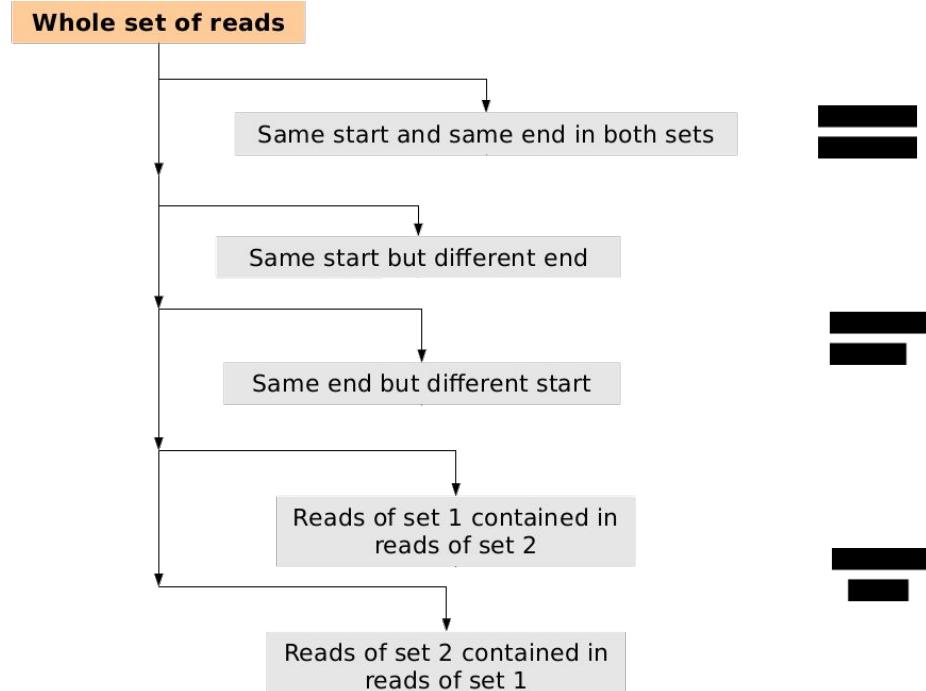


# Nucleosome Dynamics workflow



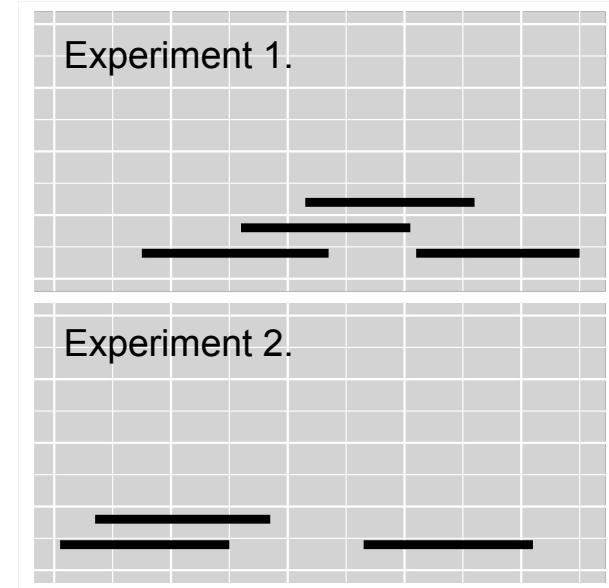
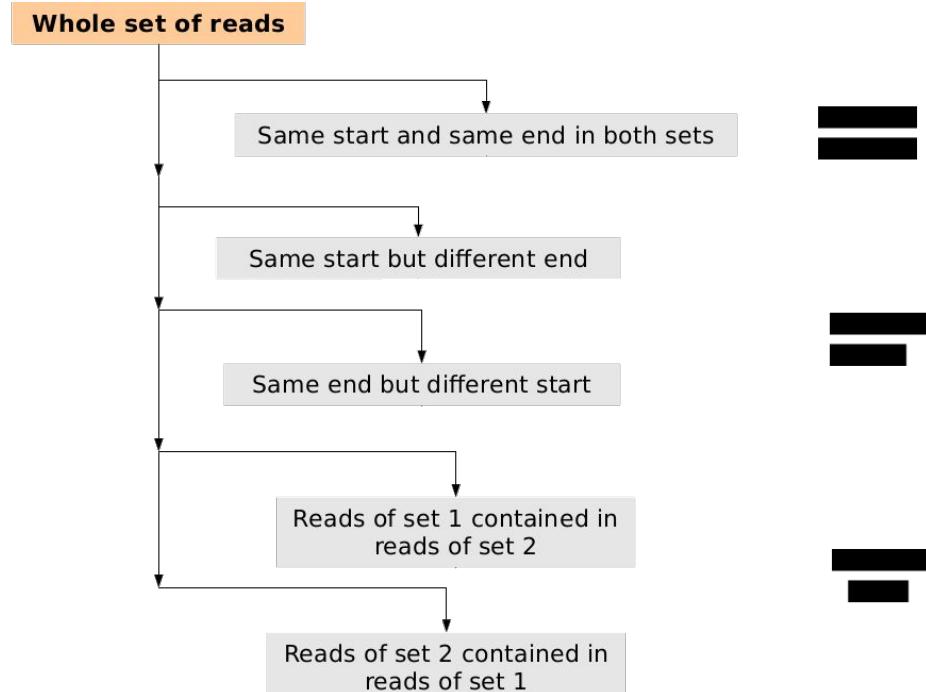


# Nucleosome Dynamics workflow



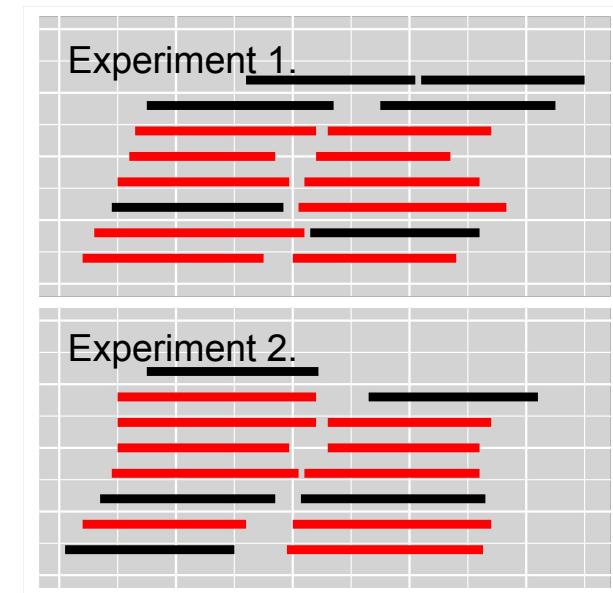
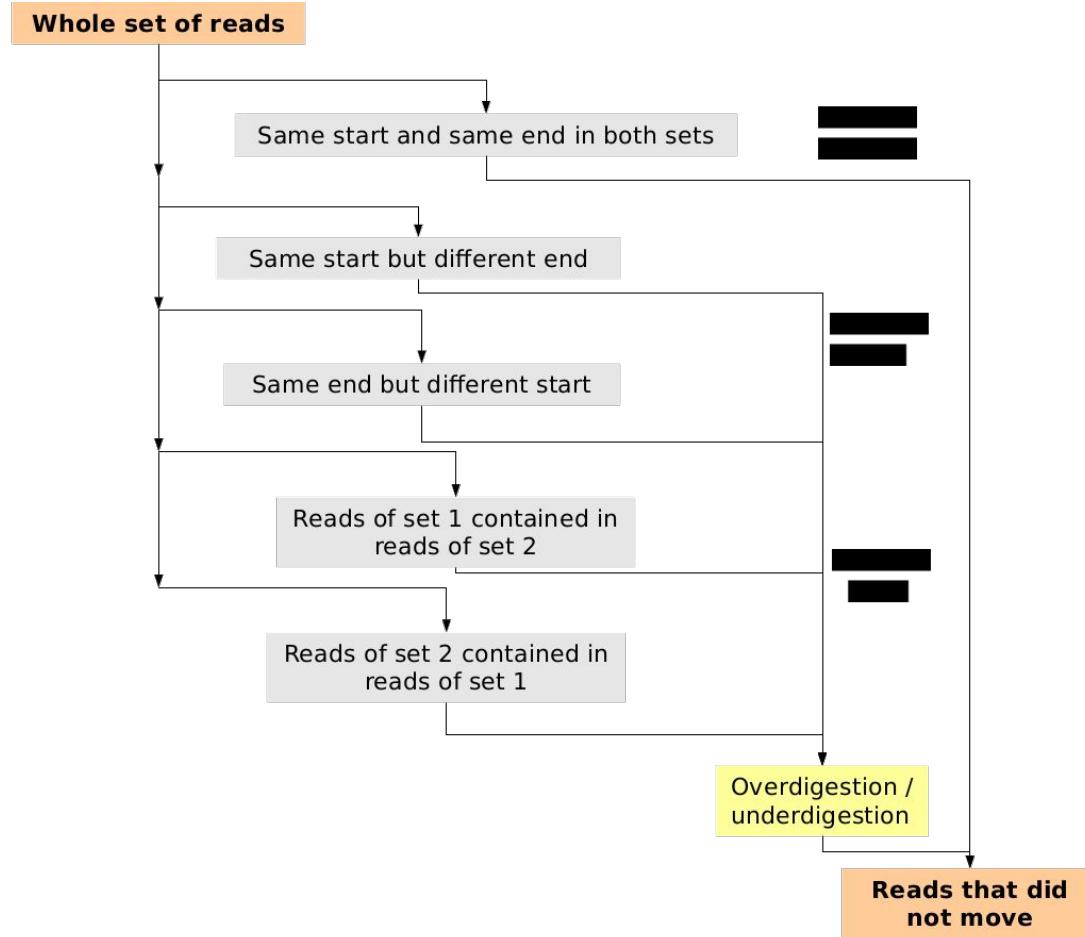


# Nucleosome Dynamics workflow



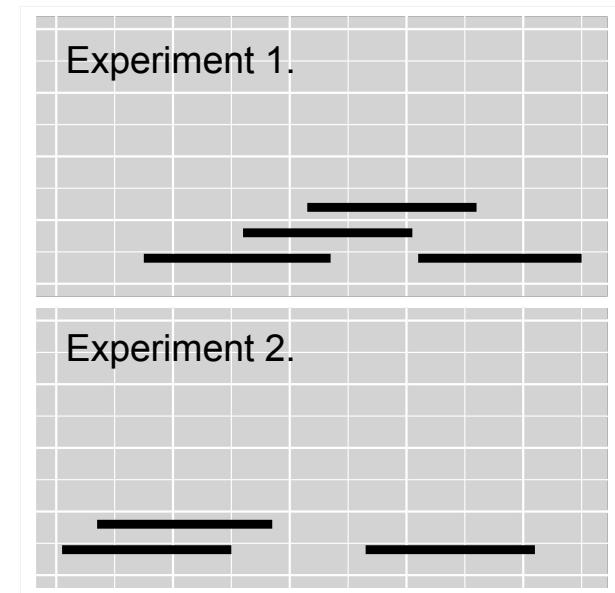
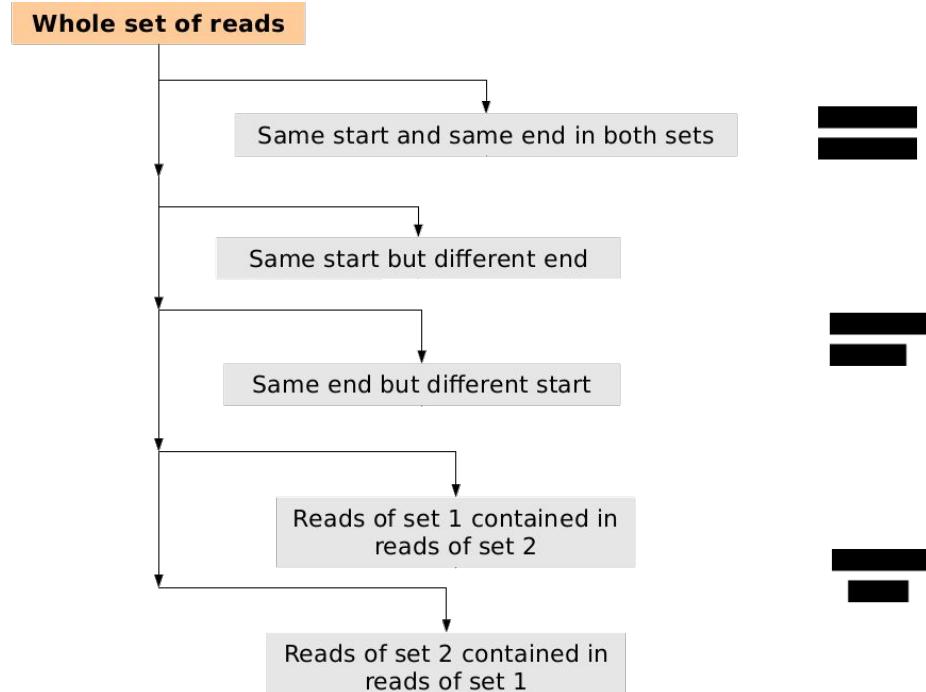


# Nucleosome Dynamics workflow



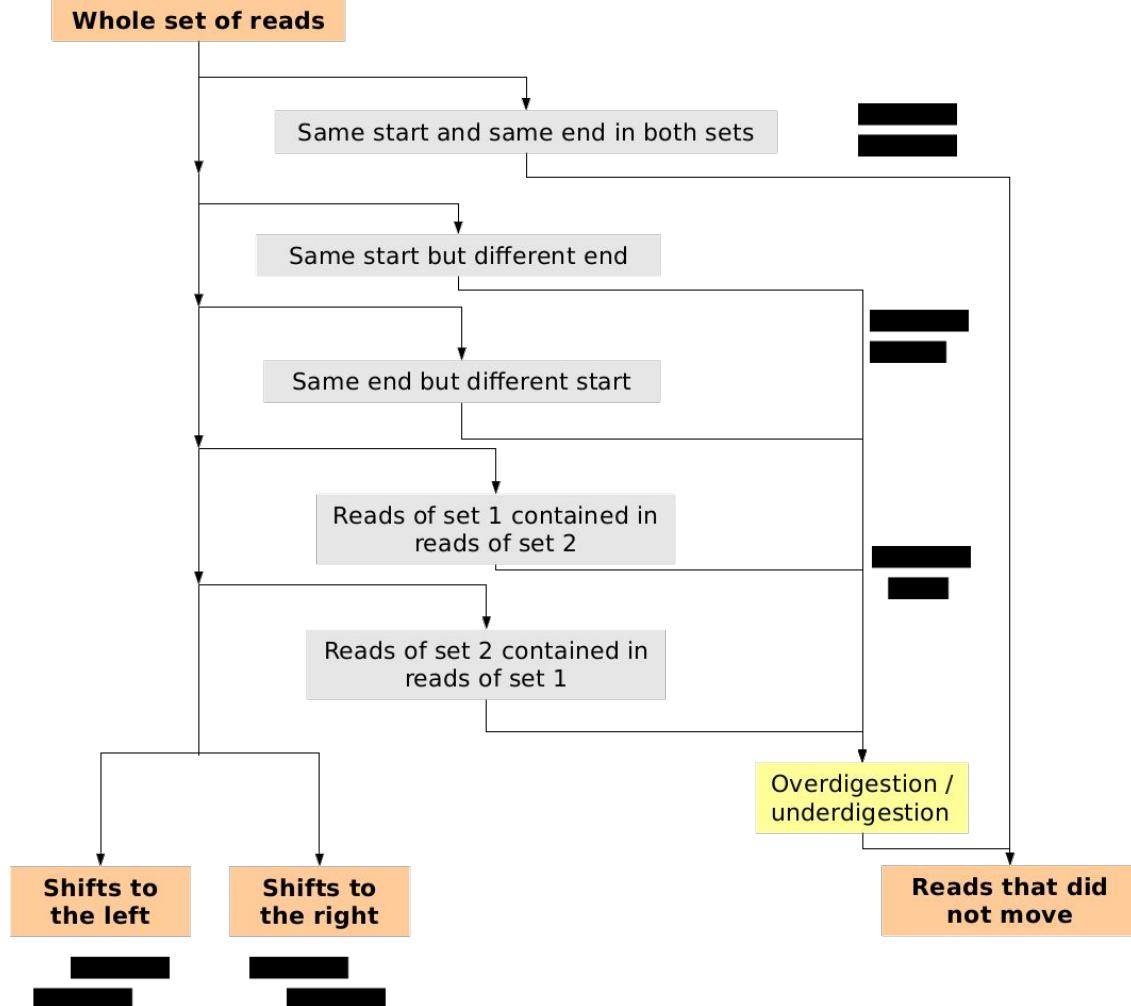


# Nucleosome Dynamics workflow





# Nucleosome Dynamics workflow



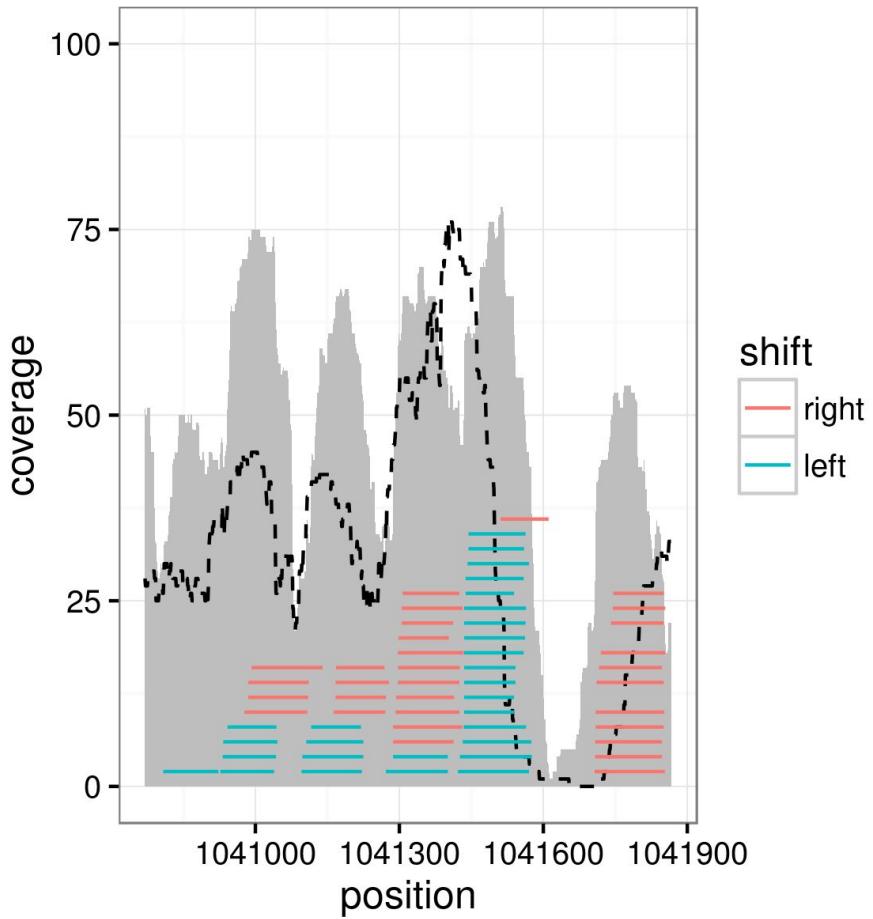
Experiment 1.

Experiment 2.

Dynamic programming  
algorithm



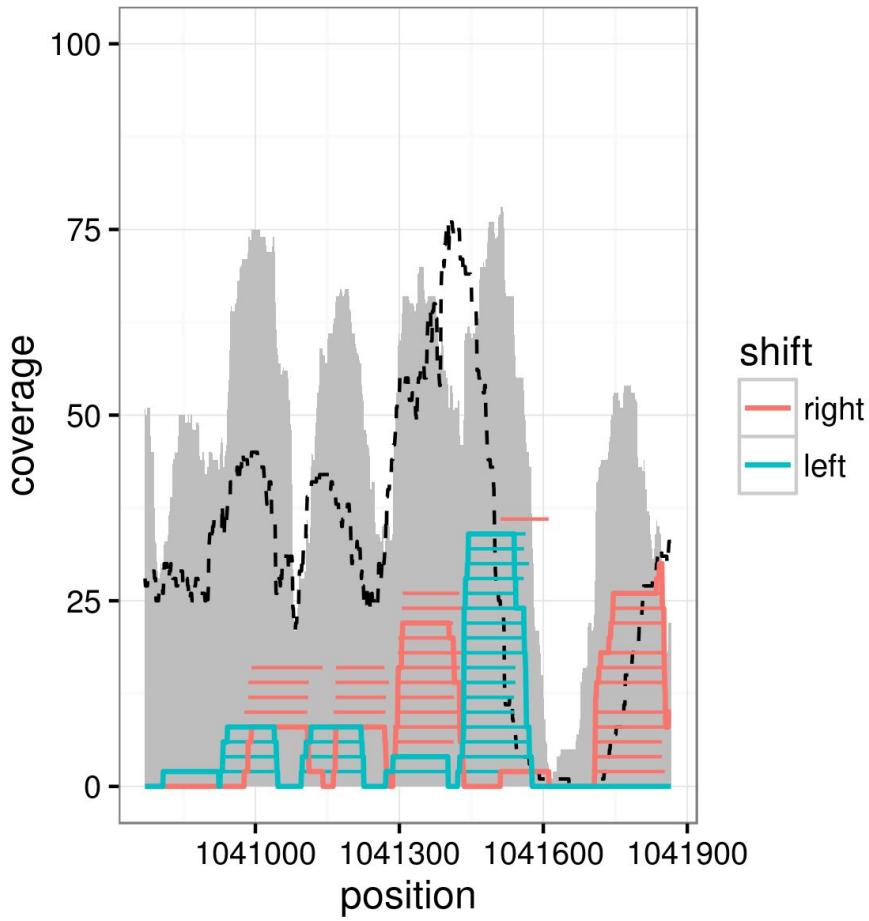
# Hotspot finding



We start with the classified reads



# Hotspot finding

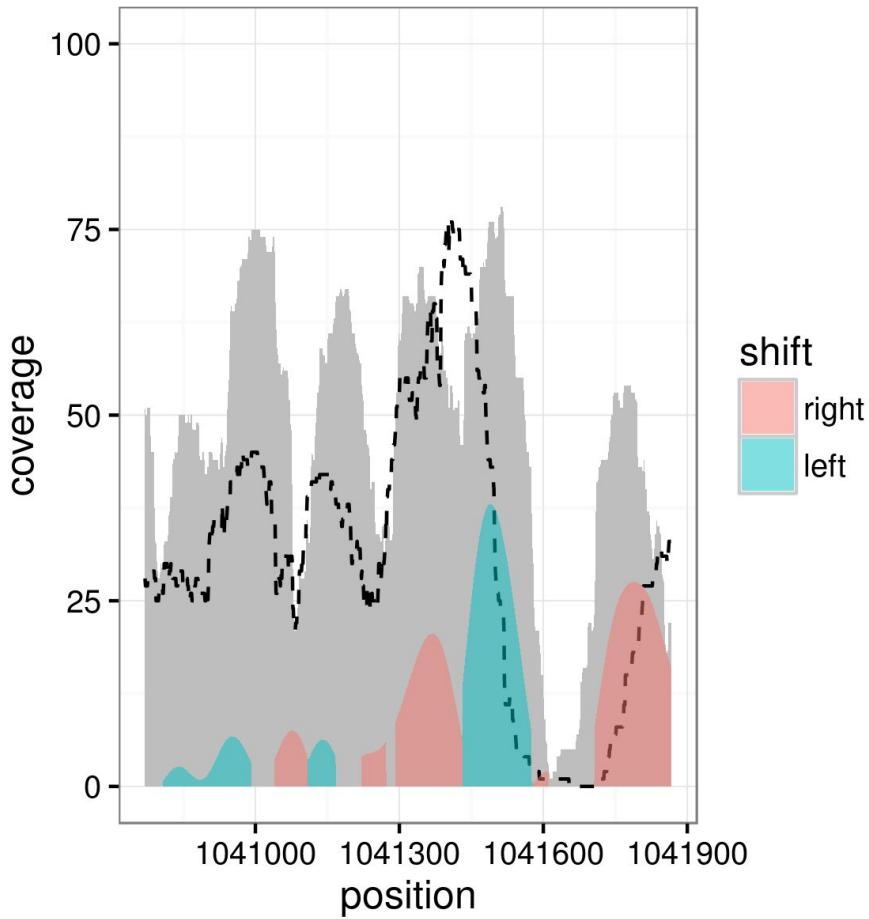


We start with the classified reads

Their coverage is calculated



# Hotspot finding



We start with the classified reads

Their coverage is calculated

The coverage is smoothed



# Inclusions and evictions

We normalize the number of reads in experiment 1, at a given position x, assuming an hypergeometric distribution

$$z = \frac{m - E(m)}{\sqrt{V(m)}}$$

where

$$f = \frac{M}{N}$$

$$E(m) = nf$$

$$V(m) = nf(1-f)\frac{N-n}{n-1}$$

where

N: Total reads in window

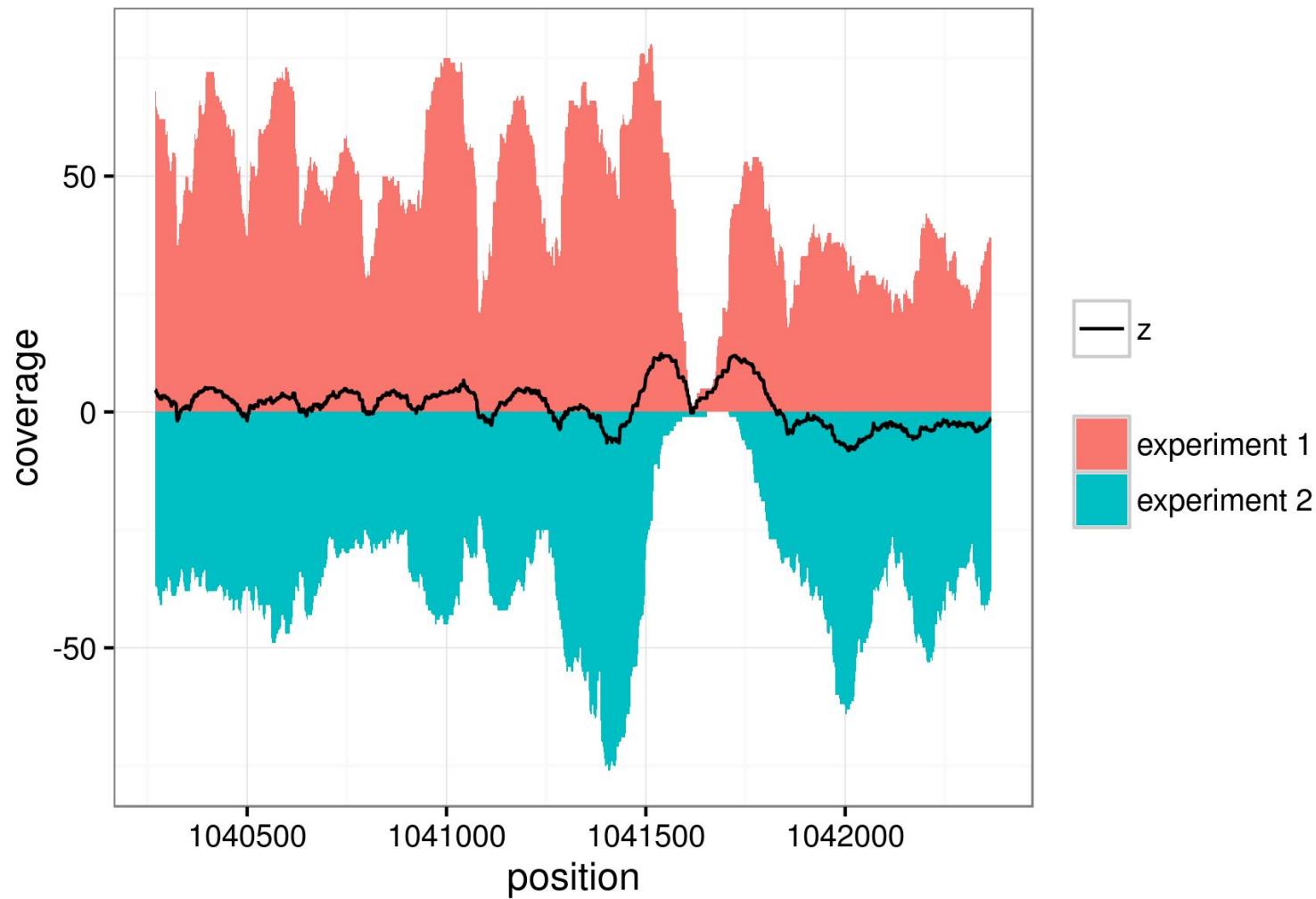
M: Reads for experiment 1 in window

n: Total reads covering position x

m: Reads covering position x in experiment 1



# Inclusions and evictions





# Hotspot scoring

We use the p-value at the peak of each hotspot using a Fisher's test

	Reads in experiment 1	Reads in experiment 2	
Reads not covering position x	$M - m$	$N - M - n + m$	$N - n$
Reads at position x	$m$	$n - m$	$n$
	$M$	$N - M$	$N$

where

$N$ : Total reads in window

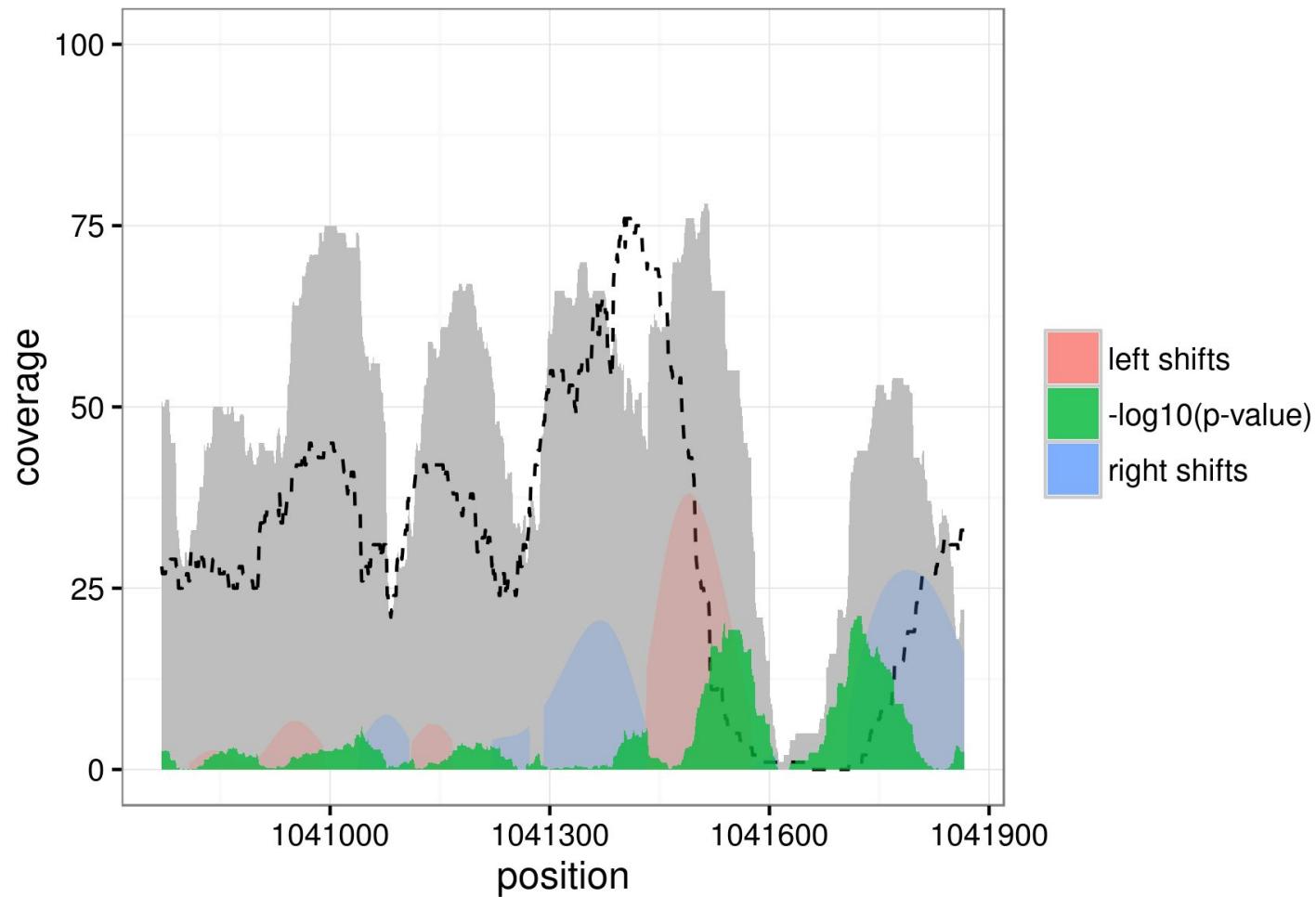
$M$ : Reads for experiment 1 in window

$n$ : Total reads covering position x

$m$ : Reads covering position x in experiment 1

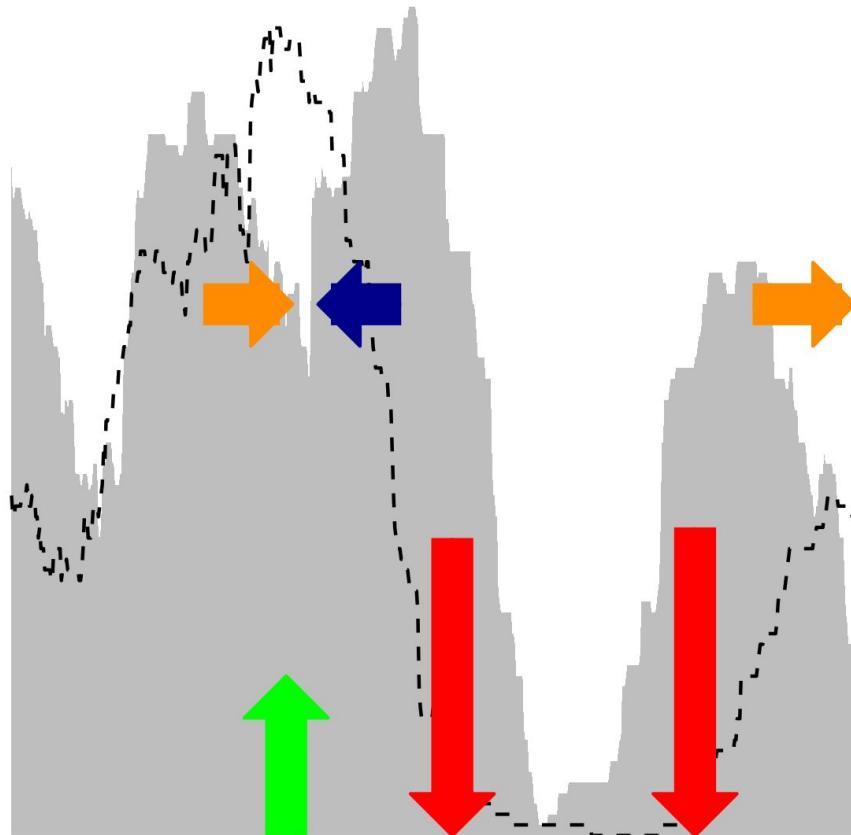


# Hotspot scoring





# Nucleosome Dynamics compares experiments





## Nucleosome-related calculations implemented

### **nucleR**: Nucleosome calling \*

- Identification of nucleosome-free regions
- Classification of TSSs
- Gaussian fitting and stiffness estimation
- Calculations on the periodicity of the coverage

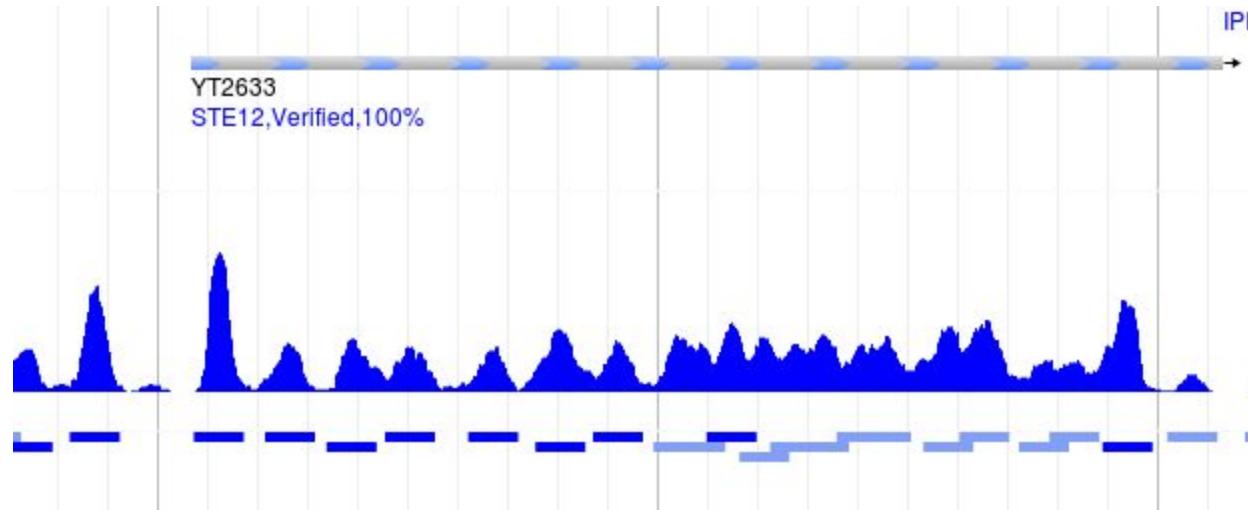
### **NucDyn**: Comparative analysis between two sets of data

*\*integrated with Chromatin Dynamics tool*



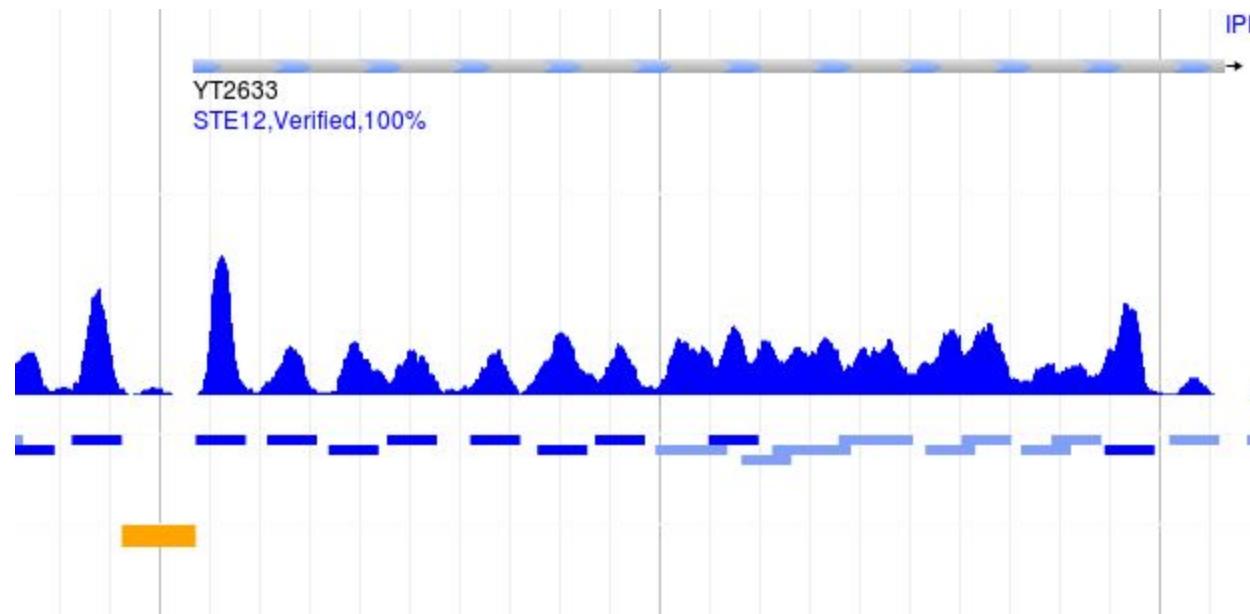


# Nucleosome calling





# Identification of nucleosome-free regions



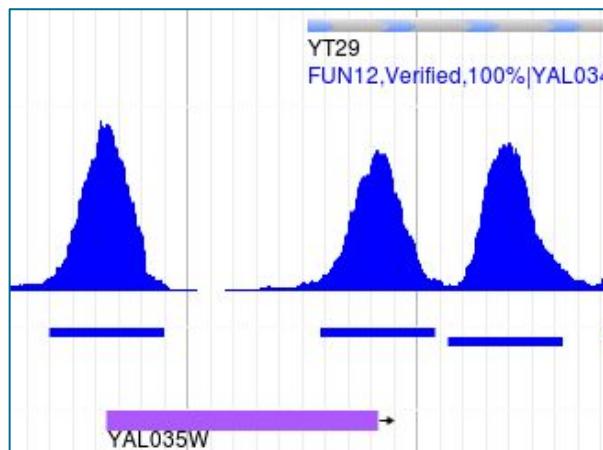


# Classification of Transcription Start Sites

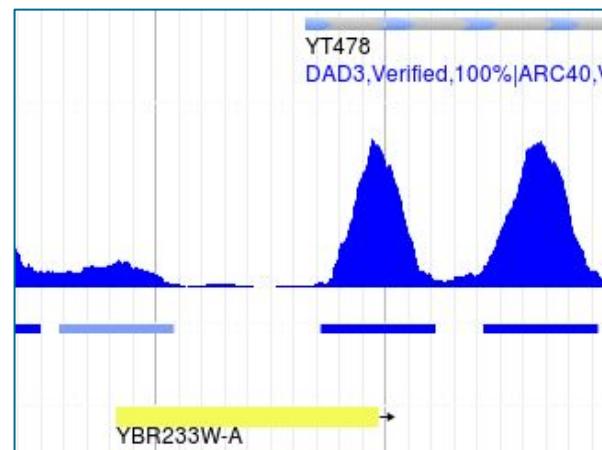
According to surrounding nucleosomes:

Well positioned / fuzzy / missing  
Open / Close

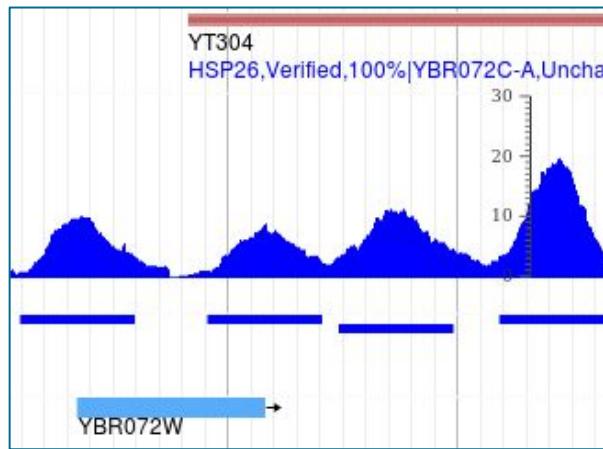
W-open-W



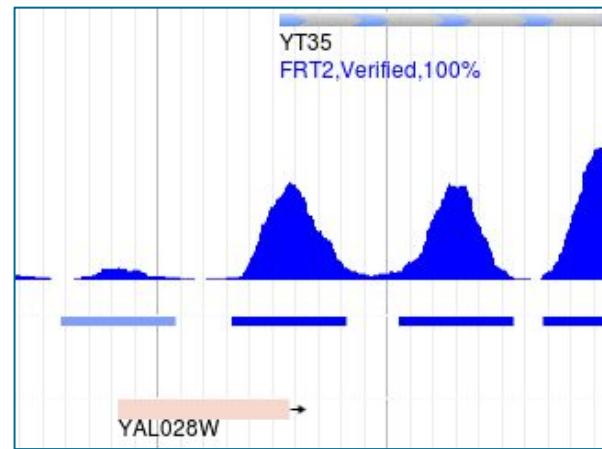
F-open-W



W-close-W

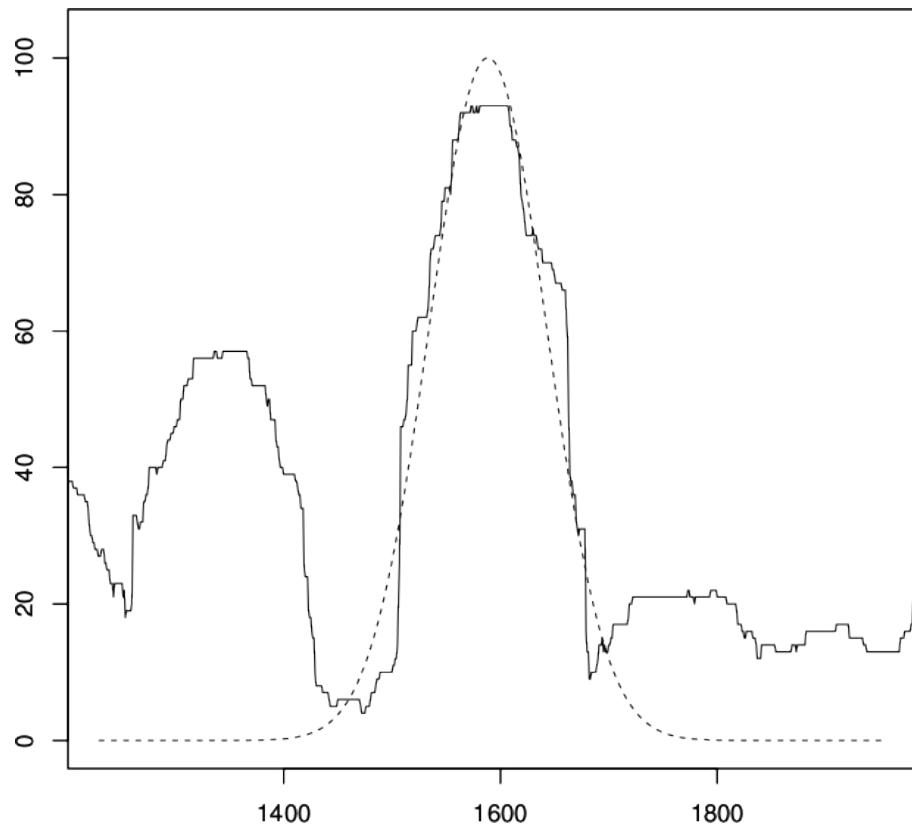


F-close-W





# Stiffness estimation



## Gaussian fitting

$$f(x) = ke^{-\frac{(x-m)^2}{2sd^2}}$$

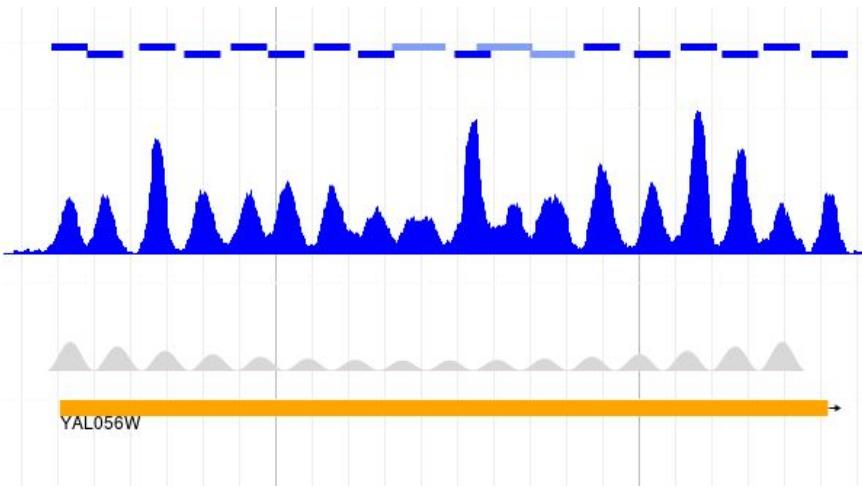
## Stiffness estimation:

$$\text{stiffness} = 2 \frac{k_B T}{sd^2}$$

(sliding propensities of  
nucleosomes)

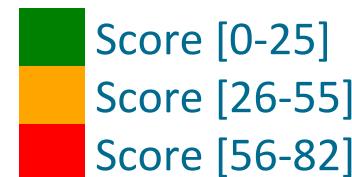


# Analyses on the periodicity



## Phasing score:

$$phase_{score} = nuc_{dist} - T \left[ \frac{nuc_{dist}}{T} \right]$$



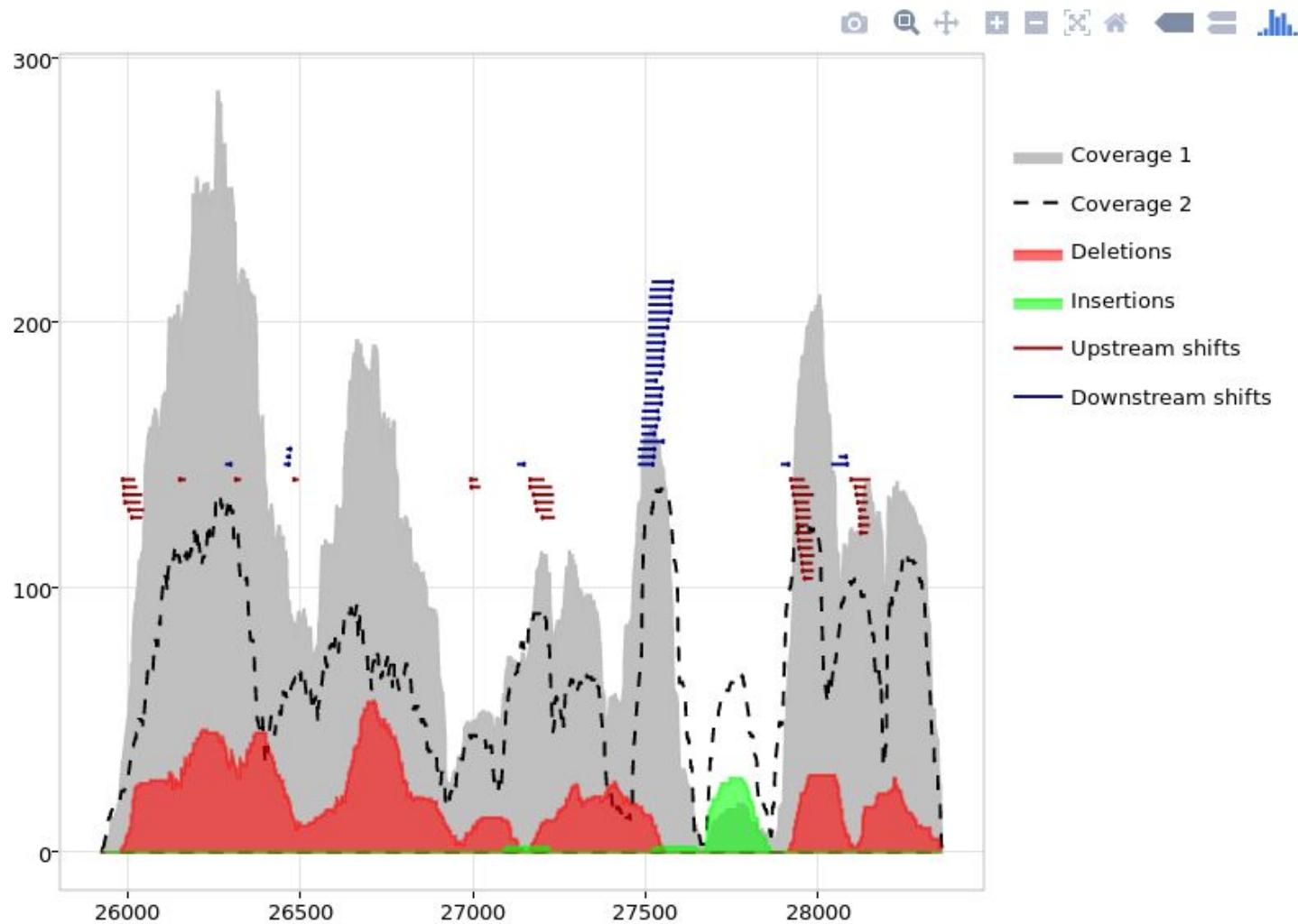
## Coverage prediction based on period:

$$R(T) = \int_{x_2}^{x_1} I(x)I(x+T)dx$$

$$autocor_{score} = \frac{R(T)}{R(0)}$$



# Comparative analysis of two sets of data





Multiscale Complex Genomics

# Nucleosome Dynamics

A tool to analyze and visualize MNase-seq data



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 676556.

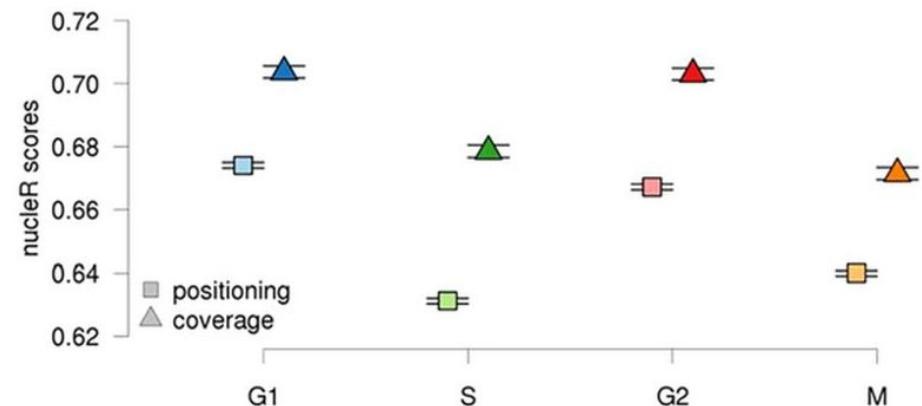
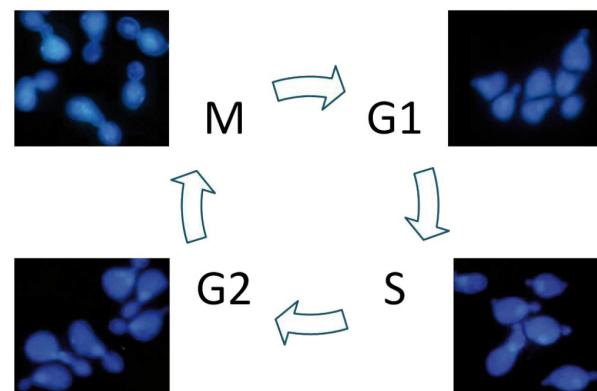




## Example: nucleosome dynamics along cell cycle

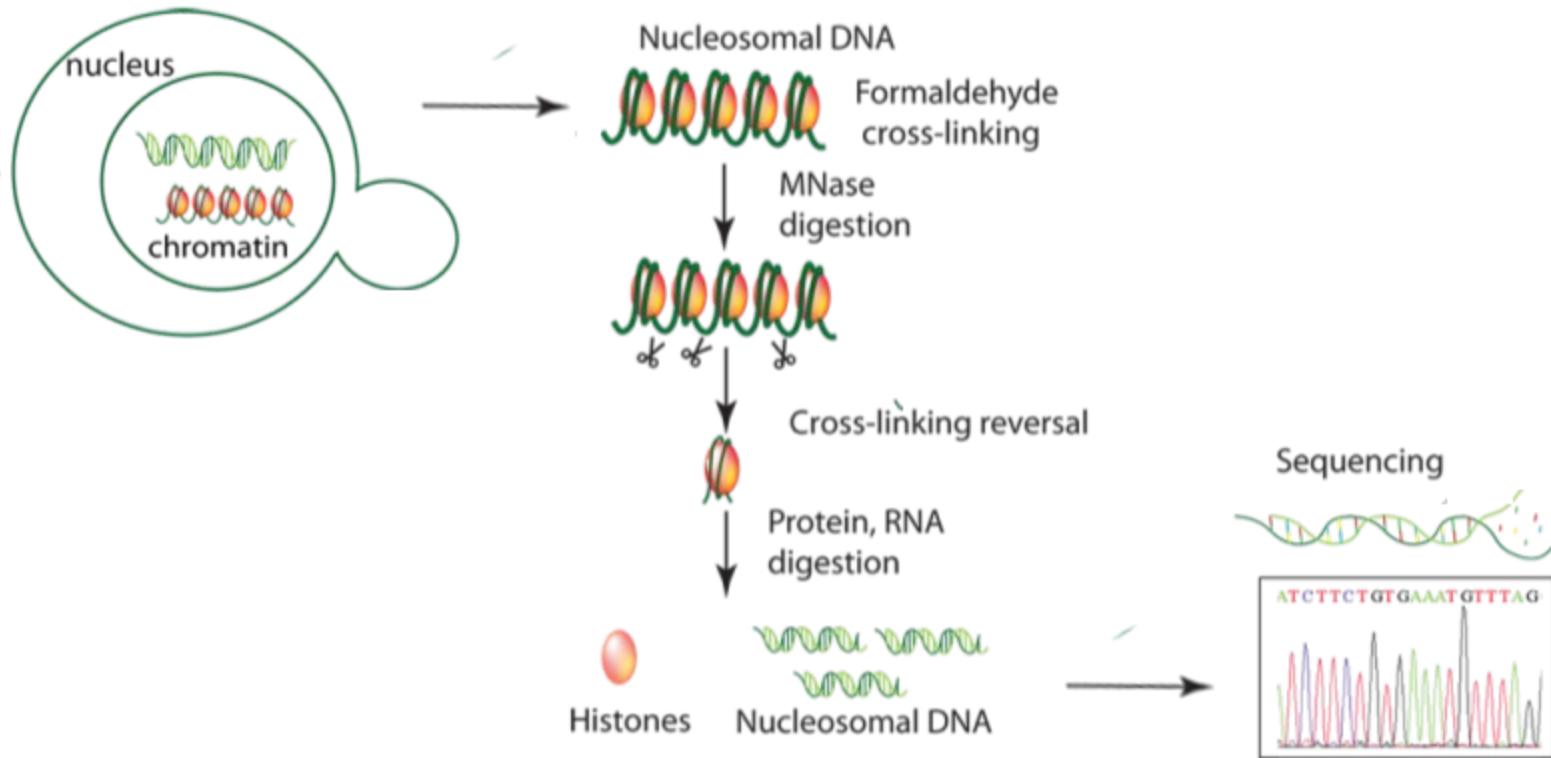
MNase-seq data from Deniz et al., Sci Rep, 2016

*Saccharomyces cerevisiae*, phases M and G2 of the cell cycle,  
chromosome II





# Input data: MNase-seq





## Input files: aligned BAM files

Software for alignment of sequences to reference genome:

- Bowtie (<http://bowtie-bio.sourceforge.net/index.shtml>)
- BWA (<http://bio-bwa.sourceforge.net/>)
- GEM (<https://github.com/smarco/gem3-mapper>)

BAM: binary format for storing sequence data (binary version of the human-readable SAM format)

It's designed to compress reasonably well



# Output files: GFF3

GFF stores genomic features in a text file

chrII	nucleaseR	Nucleosome	1532	1656	0.381059801323366	.	.	score_width=0.427010772006603;score_height=0.33510883064013;class=F
chrII	nucleaseR	Nucleosome	1635	1759	0.700576000043127	.	.	score_width=0.50554243537409;score_height=0.895609564712164;class=F
chrII	nucleaseR	Nucleosome	1823	1947	0.677492975685527	.	.	score_width=0.442129824179903;score_height=0.912856127191152;class=F
chrII	nucleaseR	Nucleosome	1948	2072	0.74205375111029	.	.	score_width=0.562846327372059;score_height=0.921261174849998;class=F
chrII	nucleaseR	Nucleosome	2075	2199	0.538017164509916	.	.	score_width=0.80855589164608;score_height=0.267478437373751;class=F
chrII	nucleaseR	Nucleosome	5790	5977	0.459863050942811	.	.	score_width=0.474565839654603;score_height=0.44516026223102;class=F
chrII	nucleaseR	Nucleosome	5972	6096	0.825998919673182	.	.	score_width=0.653043144169374;score_height=0.998954695176989;class=W
chrII	nucleaseR	Nucleosome	6130	6254	0.772606715056111	.	.	score_width=0.555086402391402;score_height=0.990127027720819;class=F
chrII	nucleaseR	Nucleosome	6251	6375	0.124549659302677	.	.	score_width=0.112638662189049;score_height=0.136460656416305;class=F
chrII	nucleaseR	Nucleosome	6402	6526	0.474968141257508	.	.	score_width=0.750715452626011;score_height=0.199220829889004;class=F
chrII	nucleaseR	Nucleosome	6518	6642	0.16332486971903	.	.	score_width=0.187044926284037;score_height=0.139604813154023;class=F
chrII	nucleaseR	Nucleosome	6611	6735	0.801868400286643	.	.	score_width=0.70871803370729;score_height=0.895018767202557;class=W
chrII	nucleaseR	Nucleosome	6774	6898	0.783719044597258	.	.	score_width=0.640502134182528;score_height=0.926935955011988;class=W
chrII	nucleaseR	Nucleosome	6924	7048	0.543865222933885	.	.	score_width=0.610264136381875;score_height=0.477466309485895;class=W
chrII	nucleaseR	Nucleosome	7086	7210	0.630808033741923	.	.	score_width=0.63327988860955;score_height=0.628336178622892;class=W
chrII	nucleaseR	Nucleosome	7313	7437	0.645858856423432	.	.	score_width=0.824399241592476;score_height=0.467318471254387;class=W
chrII	nucleaseR	Nucleosome	7542	7725	0.369902053170786	.	.	score_width=0.552278615300555;score_height=0.187525491041018;class=F
chrII	nucleaseR	Nucleosome	8130	8254	0.558292251311542	.	.	score_width=0.988377026273261;score_height=0.128207476349824;class=F
chrII	nucleaseR	Nucleosome	8304	8428	0.309057470952047	.	.	score_width=0.369355386285666;score_height=0.248759555618429;class=F
chrII	nucleaseR	Nucleosome	8398	8522	0.80569655927562	.	.	score_width=0.622435989548512;score_height=0.988957129002728;class=W

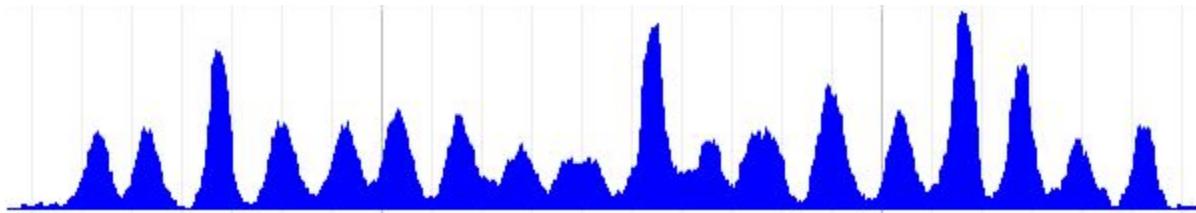




## Output files: bigWig

bigWig is the indexed binary version of the wiggle format

It is used to store and display dense continuous data





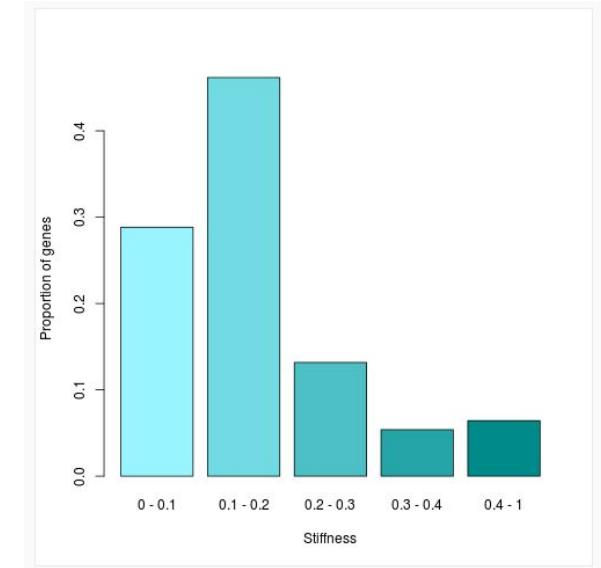
## Output files: statistics.tgz

Compressed file containing several files in png and csv format

Can be either viewed on the VRE or downloaded

Contains summaries of the calculations

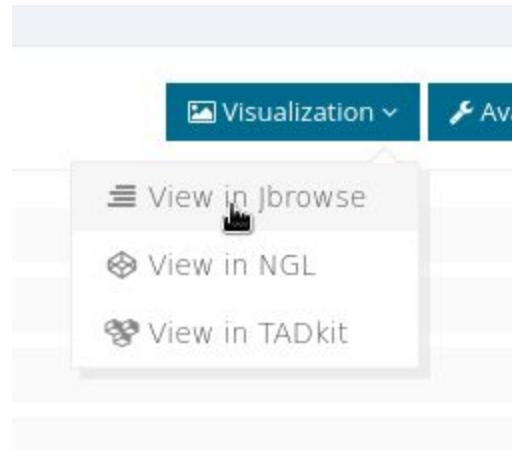
NR_G2_chrl.gff NucleR	
Class	Frequency
Fuzzy	3122
Uncertain	133
Well-positioned	1926
Total	5181





# Visualization of the results

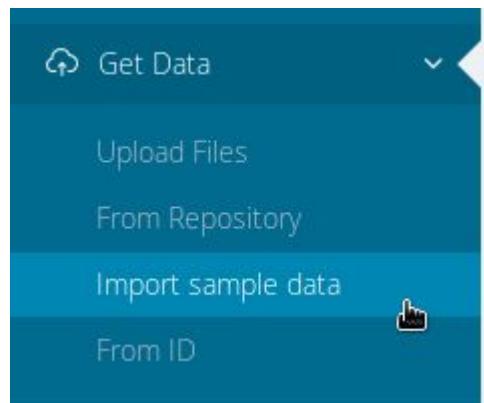
JBrowse to see the results on the genome



An HTML page with the tables for the summaries



# Sample Data



Select sample data

List of sample data

- Nucleosome Dynamics analyses
- Input and output samples for 'Nucleosome Dynamics analyses'

Import

MNase-seq data from Deniz et al., Sci Rep, 2016

*Saccharomyces cerevisiae*, phases M and G2 of the cell cycle,  
chromosome II



# Sample Data

## uploads folder: G2\_chrII.bam, M\_chrII.bam

<input type="checkbox"/>		uploads <small>i</small>	uploads	2018/09/16 19:45	83.14 M				
<input type="checkbox"/>		cellcycleG2_chrII.bam <small>i</small> BAM	MNase-Seq	uploads	2018/09/16 19:45	55.52 M			
<input type="checkbox"/>		cellcycleM_chrII.bam <small>i</small> BAM	MNase-Seq	uploads	2018/09/16 19:45	27.62 M			

## sample-NucleosomeDynamics folder: precomputed outputs

<input type="checkbox"/>		sample-Nucleoso... <small>i</small>	sample-Nucleoso...	2018/09/16 19:45	12.22 M				
<input type="checkbox"/>		ND_cellcycleG2_chr... <small>i</small> BW	Nucleosome dyna...	sample-Nucleosom...	2018/09/16 19:45	2.63 M			
<input type="checkbox"/>		ND_cellcycleG2_chr... <small>i</small> GFF3	Nucleosome dyna...	sample-Nucleosom...	2018/09/16 19:45	73.68 K			
<input type="checkbox"/>		NFR_cellcycleG2_ch... <small>i</small> GFF3	Nucleosome free re...	sample-Nucleosom...	2018/09/16 19:45	16.84 K			
<input type="checkbox"/>		NFR_cellcycleM_chr... <small>i</small> GFF3	Nucleosome free re...	sample-Nucleosom...	2018/09/16 19:45	19.84 K			
<input type="checkbox"/>		NR_cellcycleG2_chrl... <small>i</small> GFF3	Nucleosome positio...	sample-Nucleosom...	2018/09/16 19:45	615.64 K			
<input type="checkbox"/>		NR_cellcycleM_chrll... <small>i</small> GFF3	Nucleosome positio...	sample-Nucleosom...	2018/09/16 19:45	610.00 K			
<input type="checkbox"/>		P_cellcycleG2_chrll.... <small>i</small> BW	Nucleosome phasing	sample-Nucleosom...	2018/09/16 19:45	2.63 M			
<input type="checkbox"/>		P_cellcycleG2_chrll.... <small>i</small> GFF3	Nucleosome phasing	sample-Nucleosom...	2018/09/16 19:45	63.77 K			



# With only one BAM file as input

NucleR

Nucleosome Free Regions

Nucleosome Phasing

TSS Classification

Stiffness

Nucleosome positioning and peak calling

Detection of nucleosome-free regions

Analyses on the periodicity of nucleosome coverages along gene bodies

Classification of transcription start sites

Gaussian fitting and stiffness estimation



# With only one BAM file as input

<input type="checkbox"/>		uploads <small>i</small>			
<input checked="" type="checkbox"/>		cellcycleG2_chrII.bam <small>i</small> BAM		MNase-Seq	
<input type="checkbox"/>		cellcycleM_chrII.bam <small>i</small> BAM		MNase-Seq	



MANAGE FILES

Actions ▼

Visualization ▼

Available Tools ▼



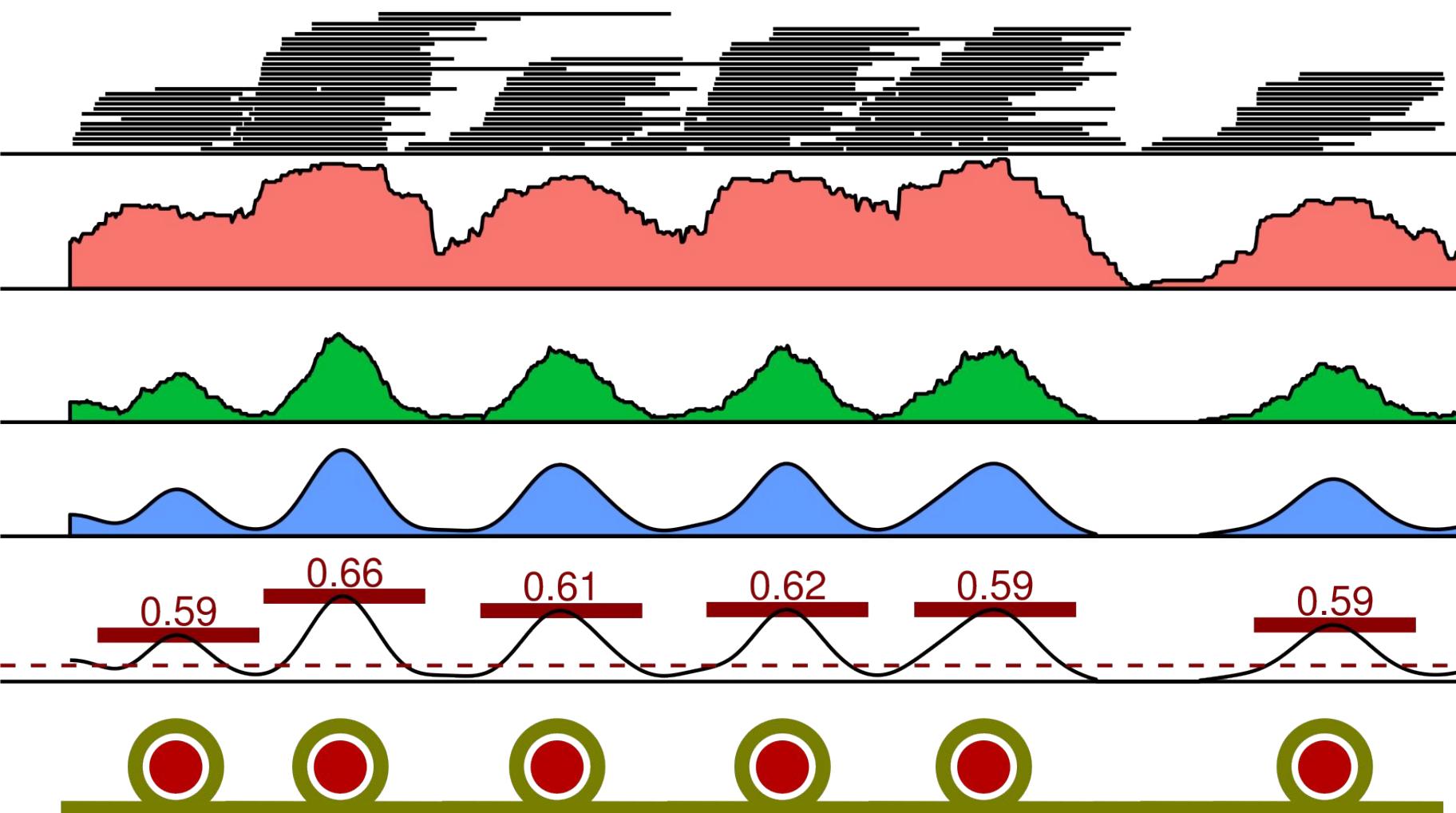
uploads / cellcycleG2\_chrII.bam i



Clear all files from list



# MNase-seq data processing using nucleR





# nucleR's parameters

ON

NucleR

▼

NucleR finds nucleosome positions from MNase experiments using Fourier transform filtering and classifies nucleosomes according to their fuzziness (more information)

MNase-seq data ②

x uploads / cellcycleG2\_chrl.bam

Width ②

147



Minimum Overlap ②

80



Dyad Length ②

50



Background level ②

35



%

Abs. Value Percentage

Height Threshold ②

0.4



Width Threshold ②

0.6



Advanced Settings

Coverage Smoothing ②

0.02





## nucleR's parameters

**Width:** size of nucleosomes in bp.

**Minimum Overlap:** nucleosomes overlapping by this number of bp or more will be merged into one.

**Dyad length:** length of the center of the nucleosome considered by the program.

**Background level:** can be an absolute value or a percentage. Used as a threshold.

**Height Threshold:** threshold on the height of the peaks to classify them as either fuzzy or well-positioned.

**Width Threshold:** threshold on the width of the peaks to classify them as either fuzzy or well-positioned.

**Coverage smoothing:** number of components to select relative to the total in the sample in the Fourier transformation smoothing.



# Nucleosome-free regions' parameters

ON  Nucleosome Free Regions ▼

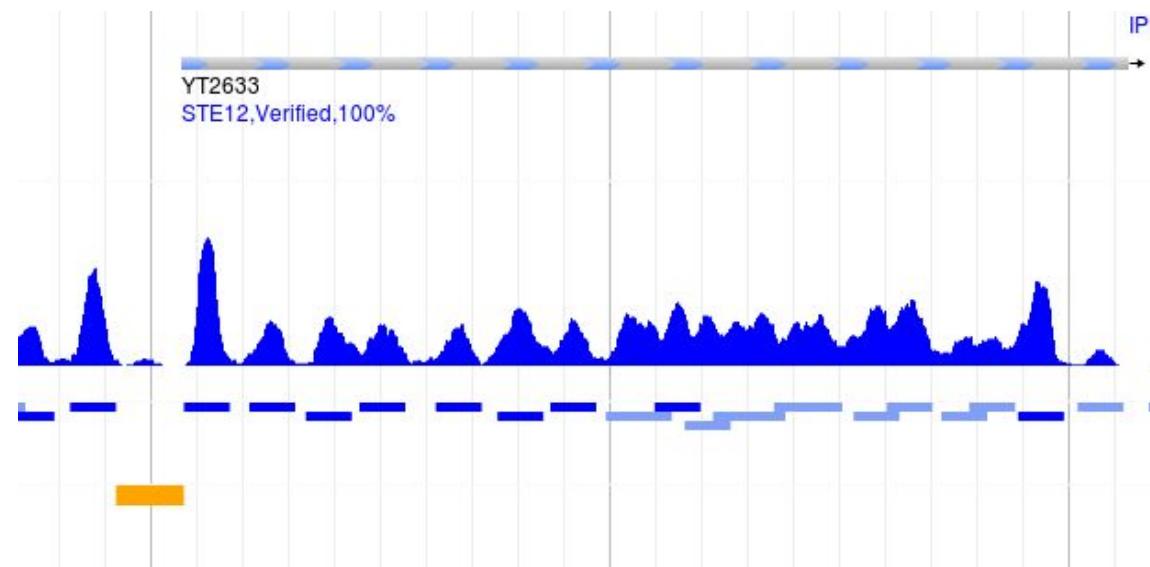
Nucleosome-free regions (NFR) are regions depleted of nucleosomes and larger than an average linker fragment. ([more information](#))

Minimum Width ?

110

Maximum width ?

400

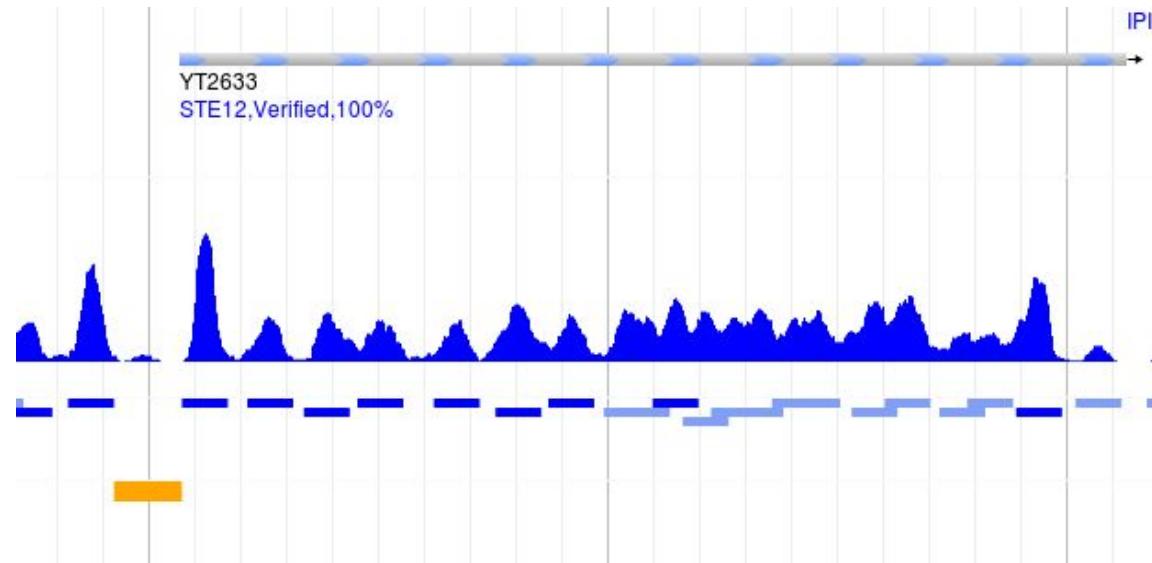




## Nucleosome-free regions' parameters

**Minimum width:** minimum length for a linked fragment to be considered a nucleosome-free region.

**Maximum width:** maximum length for a linked fragment to be considered a nucleosome-free region.



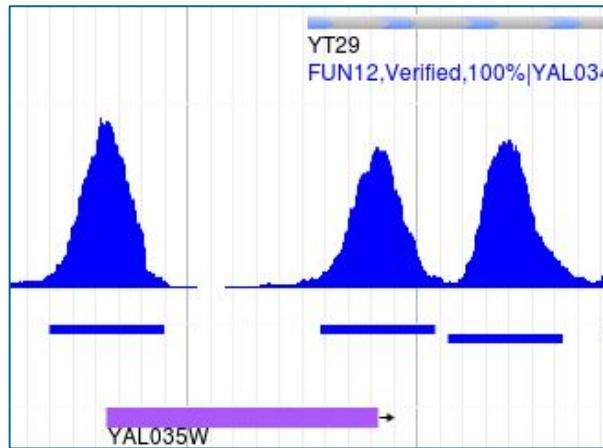


# TSS classification's parameters

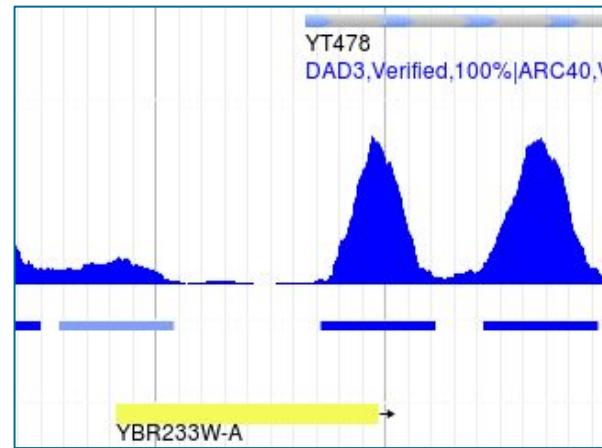
According to surrounding nucleosomes:

Well positioned / fuzzy / missing  
Open / Close

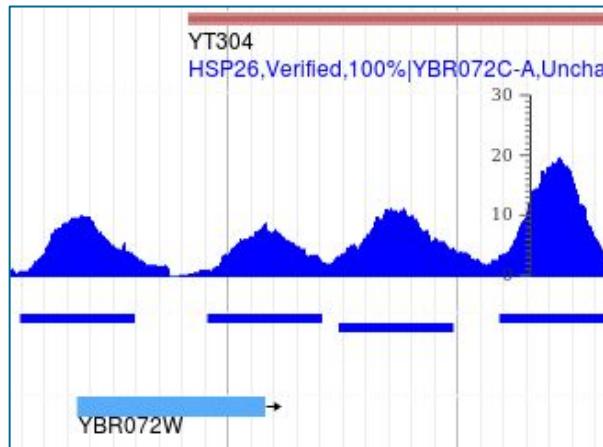
W-open-W



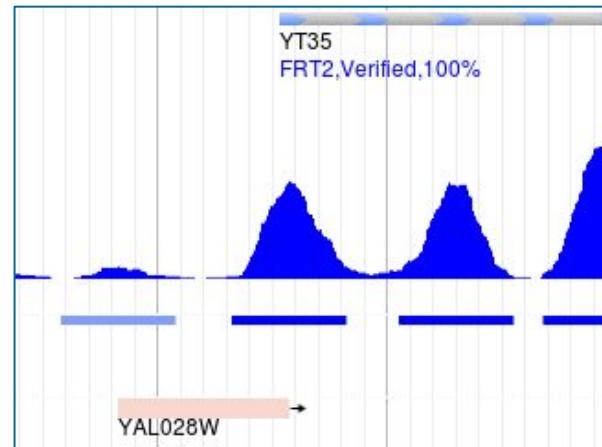
F-open-W



W-close-W



F-close-W





# TSS classification's parameters

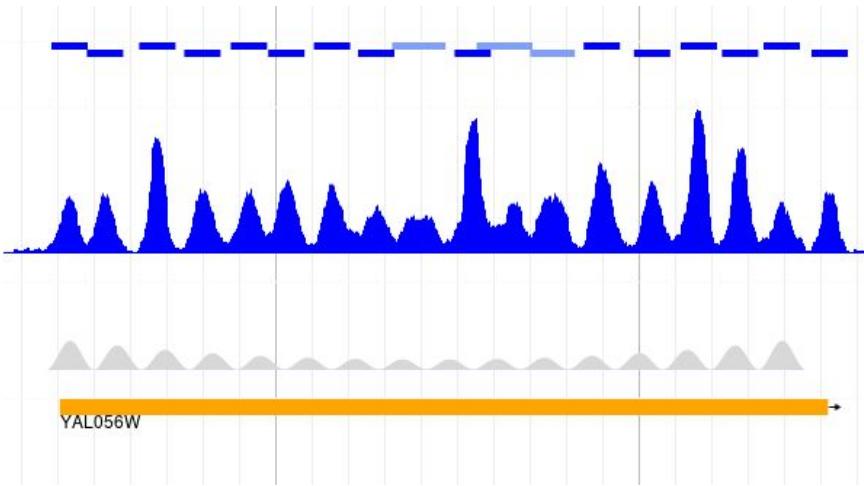
The screenshot shows a software interface titled "TSS Classification". At the top left is a "ON" button with a switch icon. Below the title, a descriptive text reads: "Classification of the Transcription Start Sites (TSS) according to the nucleosome architecture. (more information)". Underneath, there are two input fields: "Window" set to 300 and "Open threshold" set to 215. Both fields have up and down arrow buttons for adjustment.

**Window:** size of the area around the TSS considered.

**Open threshold:** distance between -1 and +1 nucleosomes to discriminate between 'open' and 'close' classes.

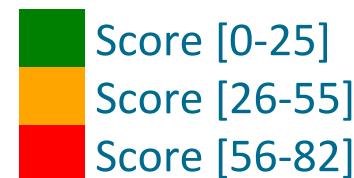


# Nucleosome phasing parameters



Phasing score:

$$phase_{score} = nuc_{dist} - T \left[ \frac{nuc_{dist}}{T} \right]$$



Coverage prediction  
based on period:

$$R(T) = \int_{x_2}^{x_1} I(x)I(x+T)dx$$

$$autocor_{score} = \frac{R(T)}{R(0)}$$



# Nucleosome phasing parameters

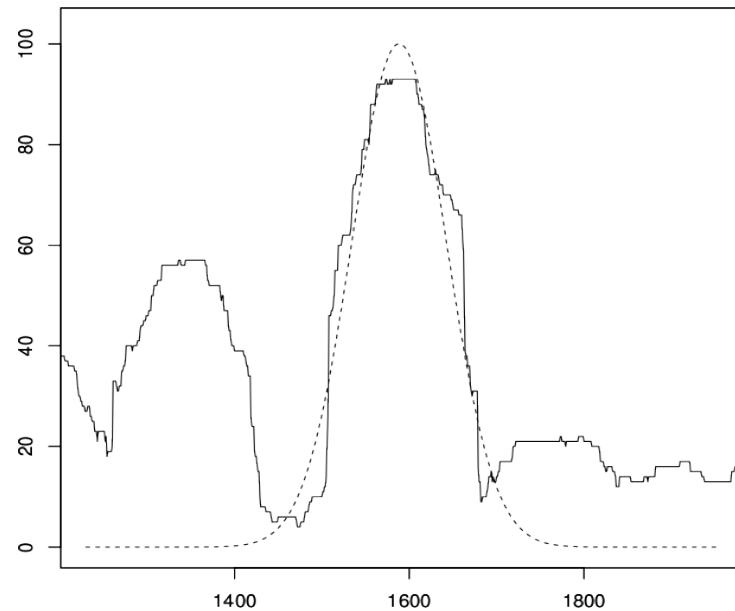
The screenshot shows a software interface with a blue header bar. On the left of the header is a white button labeled 'ON' with a small square icon. To the right of the button is the text 'Nucleosome Phasing'. On the far right of the header is a small downward-pointing arrow. The main content area has a light gray background. At the top, it says 'Nucleosome phasing along a given gene between the first and last nucleosome ([more information](#))'. Below this, there is a section labeled 'Period' with a question mark icon. A text input field contains the value '165'. To the right of the input field is a small control panel with up and down arrows.

**Period:** the assumed theoretical nucleosome periodicity

*(current default works good for yeast)*



# Stiffness parameters



## Gaussian fitting

$$f(x) = ke^{-\frac{(x-m)^2}{2sd^2}}$$

## Stiffness estimation:

$$\text{stiffness} = 2 \frac{k_B T}{sd^2}$$

(sliding propensities of nucleosomes)

ON  Stiffness ▼

Measure of the resistance of a given nucleosome to be displaced, derived from the properties of the nucleosome calls fitted into a Gaussian distribution. ([more information](#))

Genomic Range ?

All



# Output visualization

File	File type	Data type
File	All	All
G2		
NFR_cellcycleG2_chrl...	GFF3	Nucleosome free re...
NR_cellcycleG2_chrl...	GFF3	Nucleosome positio...
P_cellcycleG2_chrl...	BW	Nucleosome phasing
P_cellcycleG2_chrl...	GFF3	Nucleosome phasing
STF_cellcycleG2_chrl...	GFF3	Nucleosome stiffness
TSS_cellcycleG2_chrl...	GFF3	Nucleosome TSS

**MANAGE FILES**

Actions Visualization Available Tools

View in Jbrowse

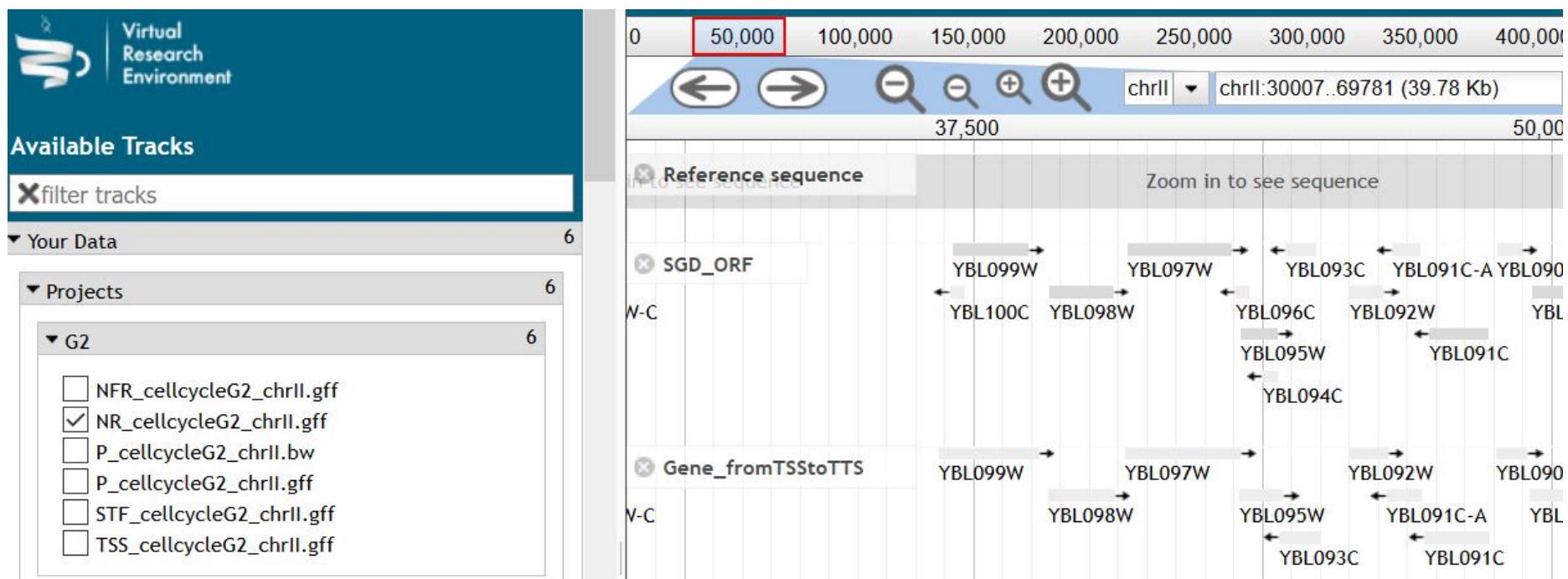
View in TADkit

G2 / NFR\_cellcycleG2\_chrl.gff   
 G2 / NR\_cellcycleG2\_chrl.gff   
 G2 / P\_cellcycleG2\_chrl.bw   
 G2 / P\_cellcycleG2\_chrl.gff   
 G2 / STF\_cellcycleG2\_chrl.gff   
 G2 / TSS\_cellcycleG2\_chrl.gff

Clear all files from list

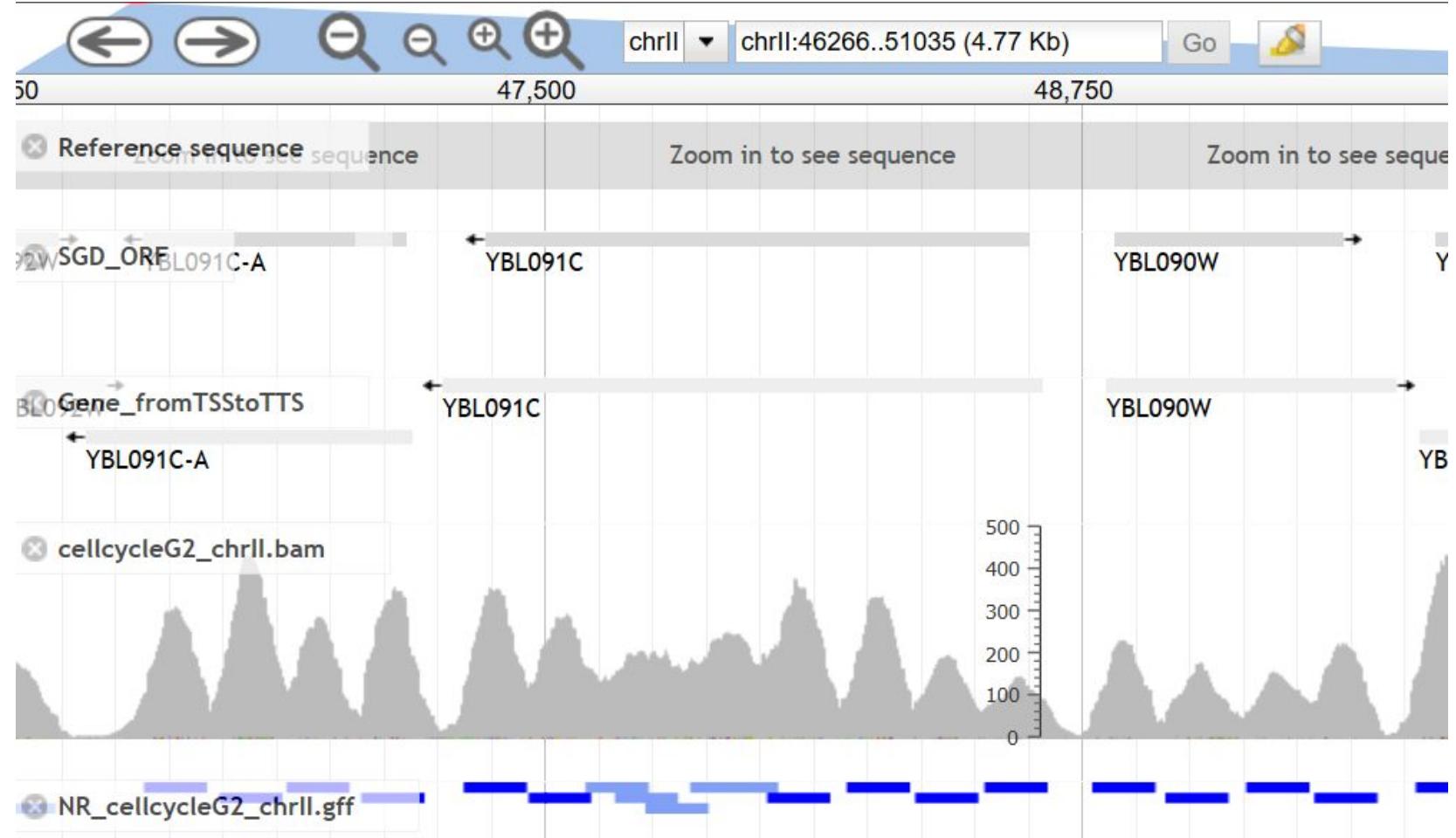


# Output visualization in JBrowse





# nucleR's outputs





# nucleR's outputs

File	File type	Data type	Project	Date	Size	Actions
<input type="checkbox"/> NR	All	All	All			<input checked="" type="checkbox"/> Clear filters
<input type="checkbox"/> NR_cellcycleG2_chrl... ⓘ	GFF3	Nucleosome posit...	sample-Nucleosom...	2018/09/16 19:45	615.64 K	

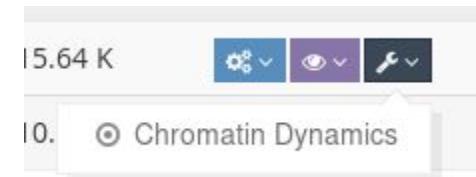
**class:** a nucleosome can be well-positioned or fuzzy

**score\_height:** score for the height between 0 and 1. The closer to 1, the higher the peak.

**score\_width:** score for the width between 0 and 1. The closer to 1, the sharper the peak

**score:** weighted sum of score\_height and score\_width.

\*Can be used as input for the Chromatin Dynamics tool





# nucleR's outputs

Nucleosome

Primary Data

Type	Nucleosome
Score	0.702139924765073
Position	chrII:41705..41851
Length	147 bp

Attributes

class	W
score_height	0.70117456993227
score_width	0.703105279597876
source	nucleR

Region sequence

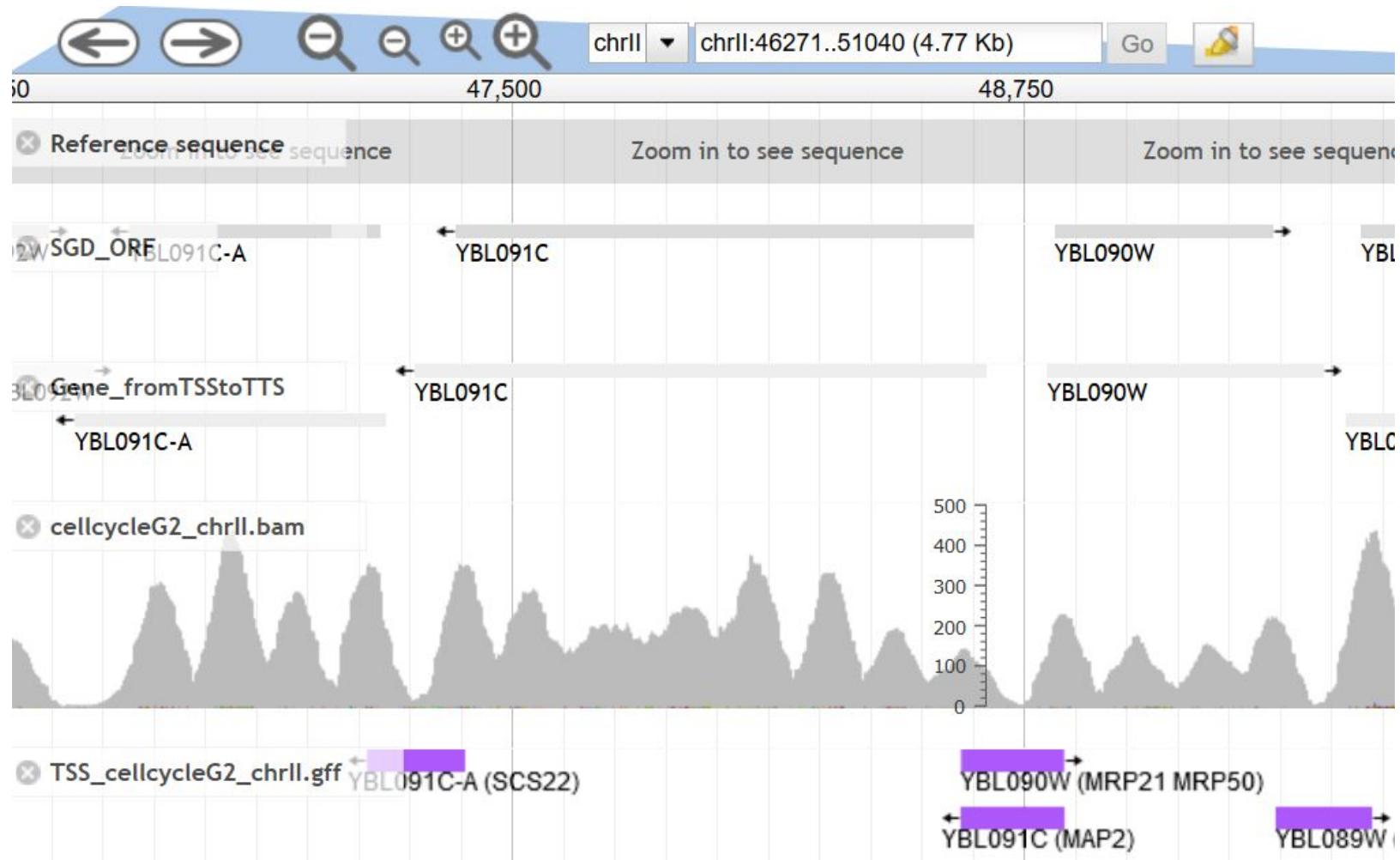
FASTA

```
>chrII chrII:41705..41851 class=Nucleosome length=147
AAATTGCGGTGTGAAATTTCAGGATCGATTGAGCAACTAAGGAACGTTGTCGAAGATATTA
ATCAAGCGAAAGACTTATCGAAAATGTTAACACAGATTGATAACTCTTAACTGAAGAAG
AATTGCAAGCAGCTGTCCCAG
```

OK



# TSS classification's outputs





# TSS classification's outputs

File	File type	Data type	Project	Date	Size	Actions
<input type="checkbox"/> tss	All	All	All			Clear filters
<input type="checkbox"/> TSS_cellcycleG2_chr... ⓘ	GFF3	Nucleosome TSS	sample-Nucleosom...	2018/09/16 19:45	64.00 K	

**classification:** class of the TSS

**distance:** distance between nucleosomes -1 and +1

**nucleosome\_minus1:** position of the nucleosome -1

**nucleosome\_plus1:** position of the nucleosome +1



# TSS classification's outputs

**TSS classification**

**Primary Data**

Name [YBL091C \(MAP2\)](#)

Type TSS classification

Position [?](#) chrII:48596..48848 (- strand)

**Attributes**

classification [?](#) W-open-W

distance [?](#) 252

nucleosome\_minus1 [?](#) 48848

nucleosome\_plus1 [?](#) 48596

source nucleR

TSS\_position [?](#) 48659

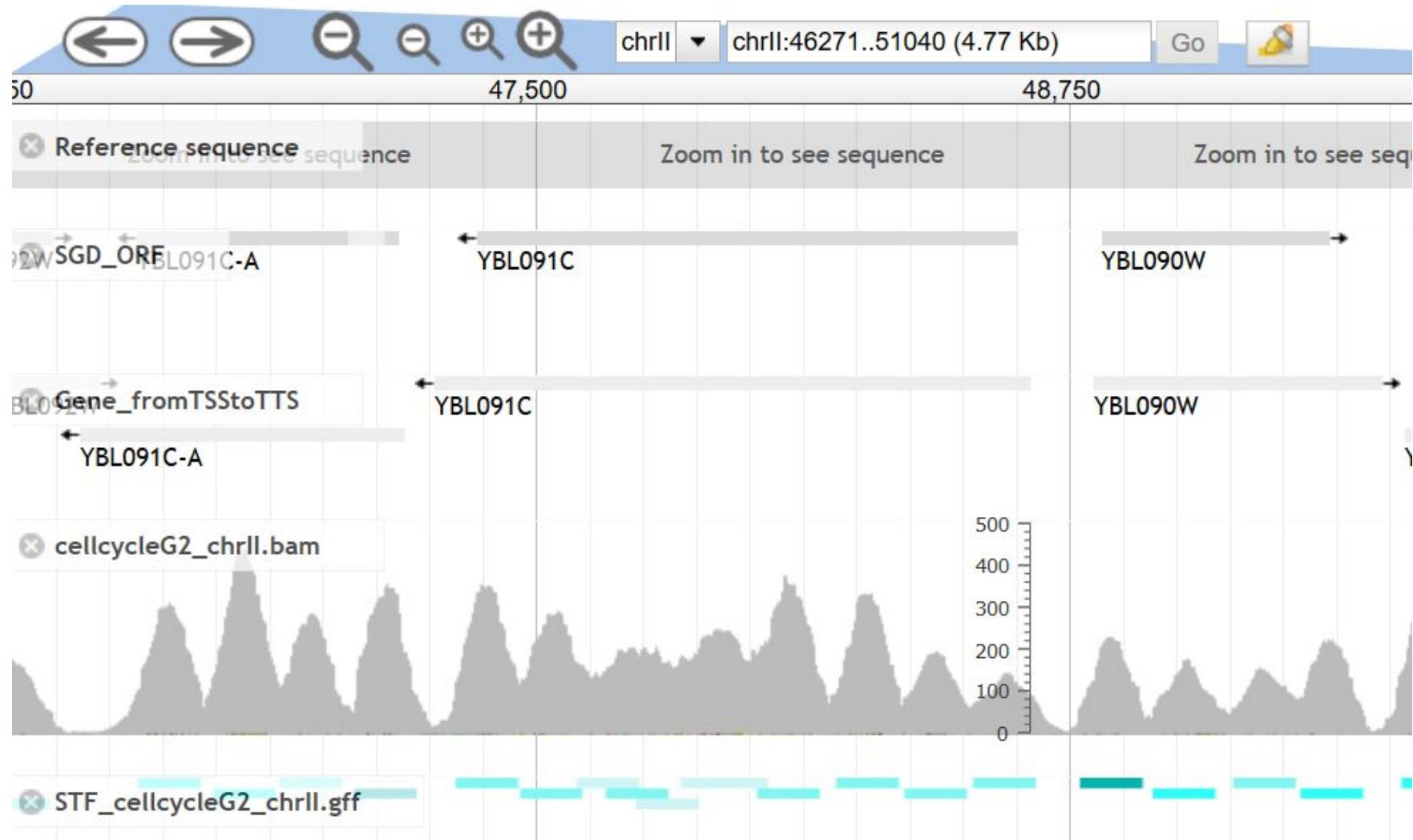
**Region sequence**

[FASTA](#)

```
>chrII chrII:48596..48848 (- strand) class=TSS classification
length=253
AAGCCTCAGCGTGCTCTCAACATTATGGTTATGCTCTTATGGGTGATATTGACAGATTTTT
ACTCTTCAAGGGACCTTAATGACCTGGTCTCACCTATTTTATTTCAATCTTGGGAAAGAA
GCGAACCTAAAAAGAAAGGAAGCCATAGCAAGAAGCTAATAAGTGGCTTTACATCAACAAG
TACTGTAGAAGCTTACCGTATTGAAAAATGACAGACGCTGAAATAGAAAATTCCCCTGCT
```



# Stiffness constant estimation's output





# Stiffness constant estimation's output

File	File type	Data type	Project	Date	Size	Actions
<input type="checkbox"/> STF	All	All	All			<input checked="" type="button"/> Clear filters
<input type="checkbox"/> STF_cellcycleG2_chr... <small>i GFF3</small>	Nucleosome stiffness	sample-Nucleosom...	2018/09/16 19:45	705.17 K		

**score:** Stiffness estimation, expressed in  $\text{kcal}\cdot\text{mol}^{-1}\cdot\text{bp}^{-1}$

**gauss\_k:** Height of the peak of the fitted gaussian curve

**gauss\_m:** Position of the peak of the gaussian curve

**gauss\_sd:** Standard deviation of the gaussian curve



# Stiffness constant estimation's output

Stiffness estimation

**Primary Data**

Type	Stiffness estimation
Score ⓘ	0.457127522579685
Position	chrII:40736..40882
Length	147 bp

**Attributes**

gauss_k ⓘ	102
gauss_m ⓘ	40811.6898148148
gauss_sd ⓘ	36.7187923055293
nucleR_class ⓘ	W
source	nucleR

**Region sequence**

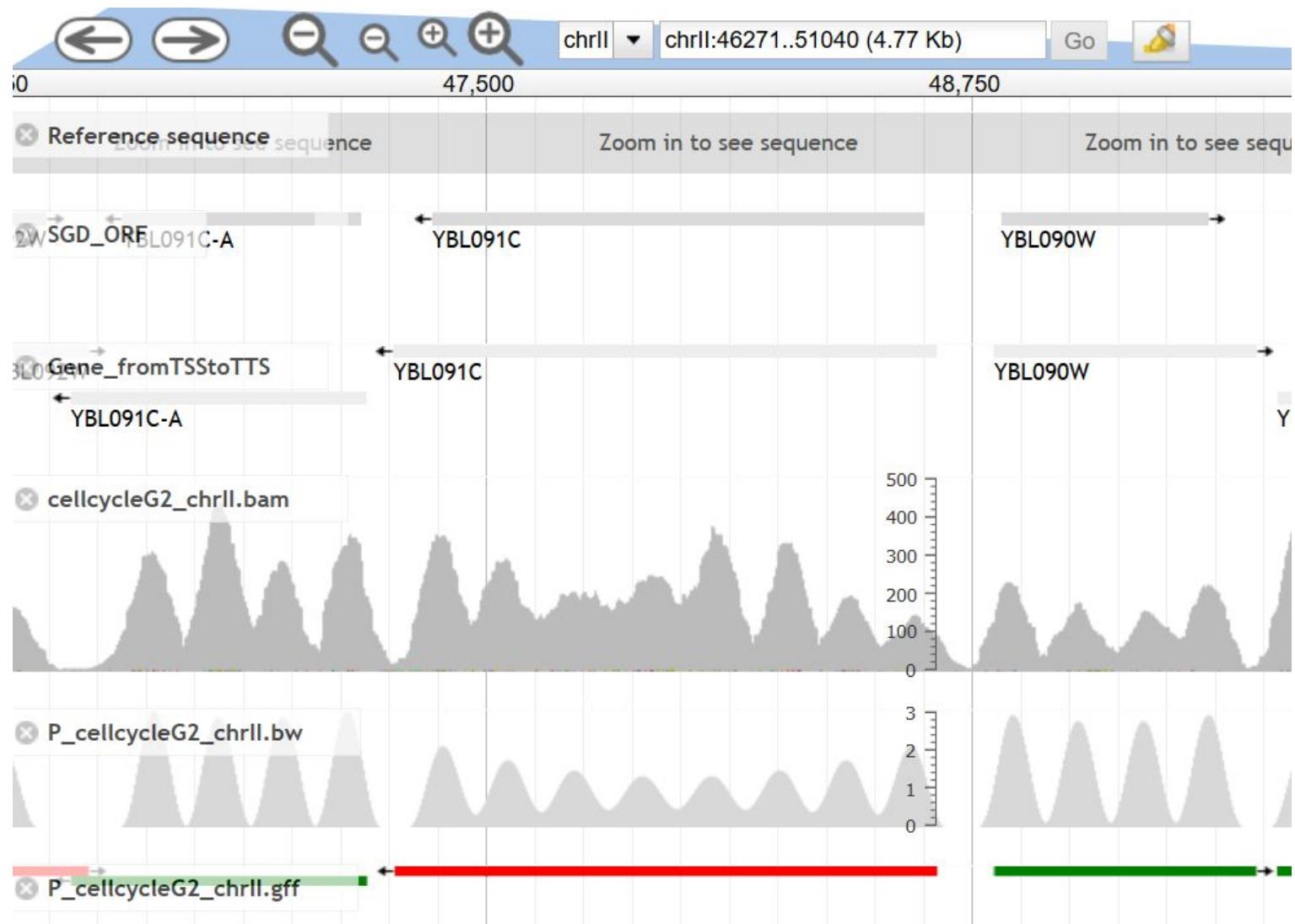
FASTA

```
>chrII chrII:40736..40882 class=Stiffness estimation length=147
TTTATATTGGCTAGTTAAATACTCTATTATTGCACCAAAAAATCATCTTAGTGGACTTTG
GAGCAAAAAAAAACAGCAGGTAGTTATATAAGATGACTACACAACTAAGGTATGAAAATAACG
ATGACGATGAAAGAGTAGAAT
```

OK



# Nucleosome phasing outputs





# Nucleosome phasing outputs

File	File type	Data type	Project	Date	Size	Actions
<input type="checkbox"/> P_	All	All	All			<input checked="" type="checkbox"/> Clear filters
<input type="checkbox"/> P_cellcycleG2_chrl....	BW	Nucleosome phasing	sample-Nucleosom...	2018/09/16 19:45	2.63 M	
<input type="checkbox"/> P_cellcycleG2_chrl....	GFF3	Nucleosome phasing	sample-Nucleosom...	2018/09/16 19:45	63.77 K	

## GFF:

**nucleosome\_first:** position of the first nucleosome in the gene

**nucleosome\_last:** position of the last nucleosome in the gene

**score\_autocorrelation:** autocorrelation of the coverage with itself shifted one period

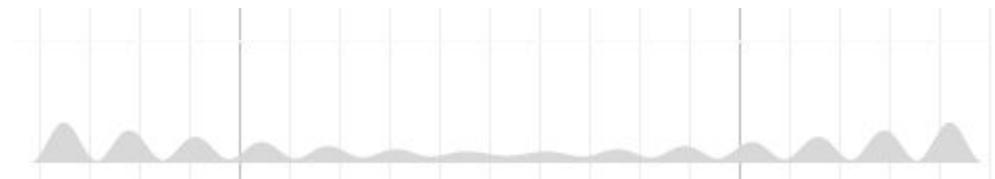
**score\_phase:** phase between first and last nucleosome

## bigWig:

Theoretical periodical coverage calculated using first and last nucleosome positions



# Nucleosome phasing outputs





# Results: statistics summarizing the calculations



<input type="checkbox"/>	<input type="checkbox"/>	sample-Nucleoso... <a href="#">i</a>		sample-Nucleoso... <a href="#">i</a>	2018/09/16 19:45	12.22 M	<a href="#">⚙️</a>	<a href="#">eye</a>
<input type="checkbox"/>		ND_cellcycleG2_chr... <a href="#">i</a>	BW	Nucleosome dyna...	sample-Nucleosom...	2018/09/16 19:45	<a href="#">View Results</a>	



# Results: statistics summarizing the calculations

<a href="#"> Download all in a compressed tar.gz file</a>
ND_G2_chrlI_M_chrlI.gff Nucleosome Dynamics
NFR_G2_chrlI.gff Nucleosome Free Regions
NFR_M_chrlI.gff Nucleosome Free Regions
NR_G2_chrlI.gff NucleR
NR_M_chrlI.gff NucleR
P_G2_chrlI.gff Phasing
P_M_chrlI.gff Phasing
STF_G2_chrlI.gff Stiffness
STF_M_chrlI.gff Stiffness
TSS_G2_chrlI.gff TSS Classification
TSS_M_chrlI.gff TSS Classification



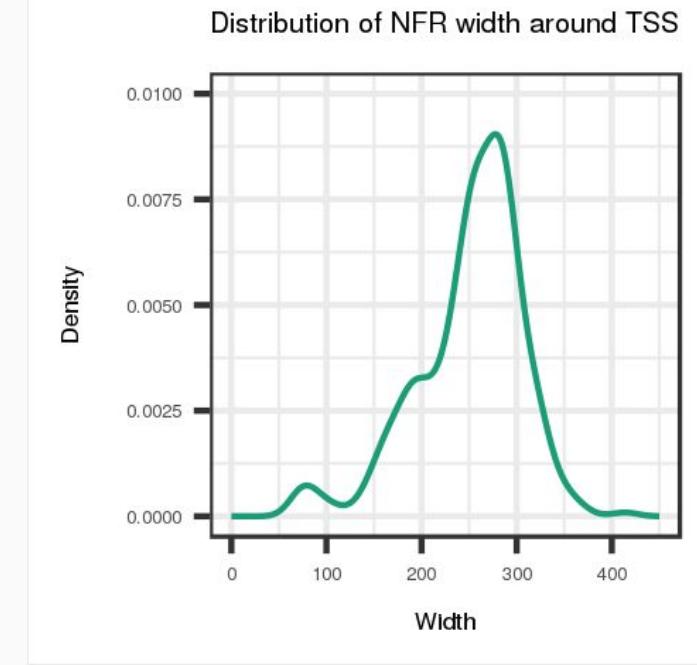
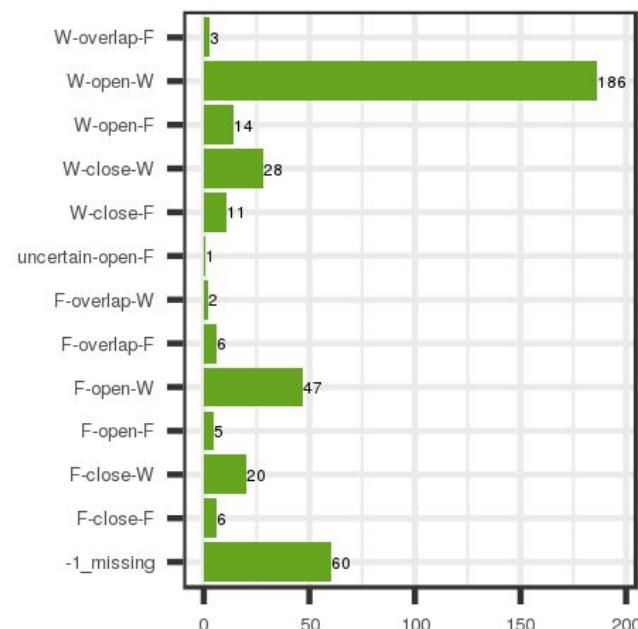
# NucleR's statistics

NR_cellcycleG2_chrl.gff NucleR	
Class	Frequency
Fuzzy	2324
Uncertain	24
Well-positioned	2559
Total	4907



# Transcription Start Sites classification's statistics

TSS\_cellcycleG2\_chrl.gff TSS Classification





# Nucleosome Free Regions' statistics

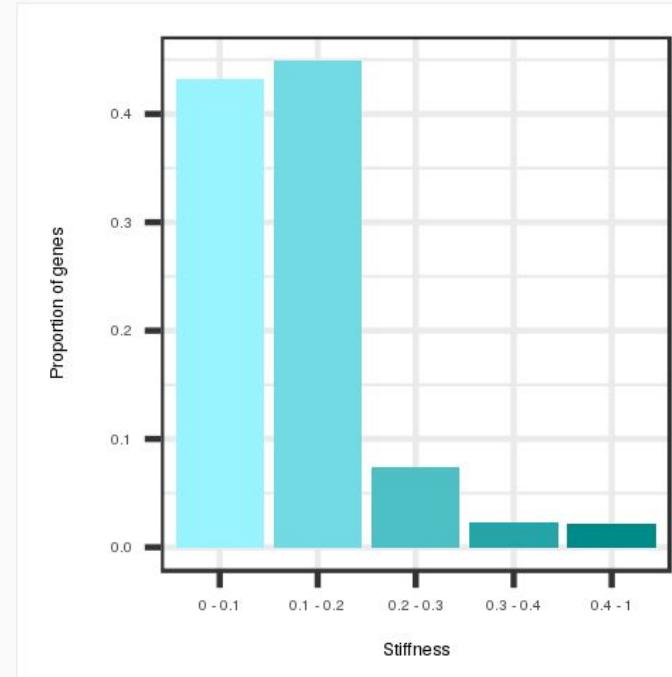
NFR_celccycleG2_chrl1.gff Nucleosome Free Regions	
NFR	Value
Total	304
Mean width	258.39
Std. Dev. width	505.84



# Stiffness constant estimation's statistics

STF\_cellcycleG2\_chrl.gff Stiffness

Statistic	Value
Mean stiffness	0.1347
Std. Dev. stiffness	0.1105





# Nucleosome phasing statistics

P_cellcycleG2_chrlI.gff Phasing	
Type	Count
Phased genes	109
Not-phased genes	137
Other genes	142

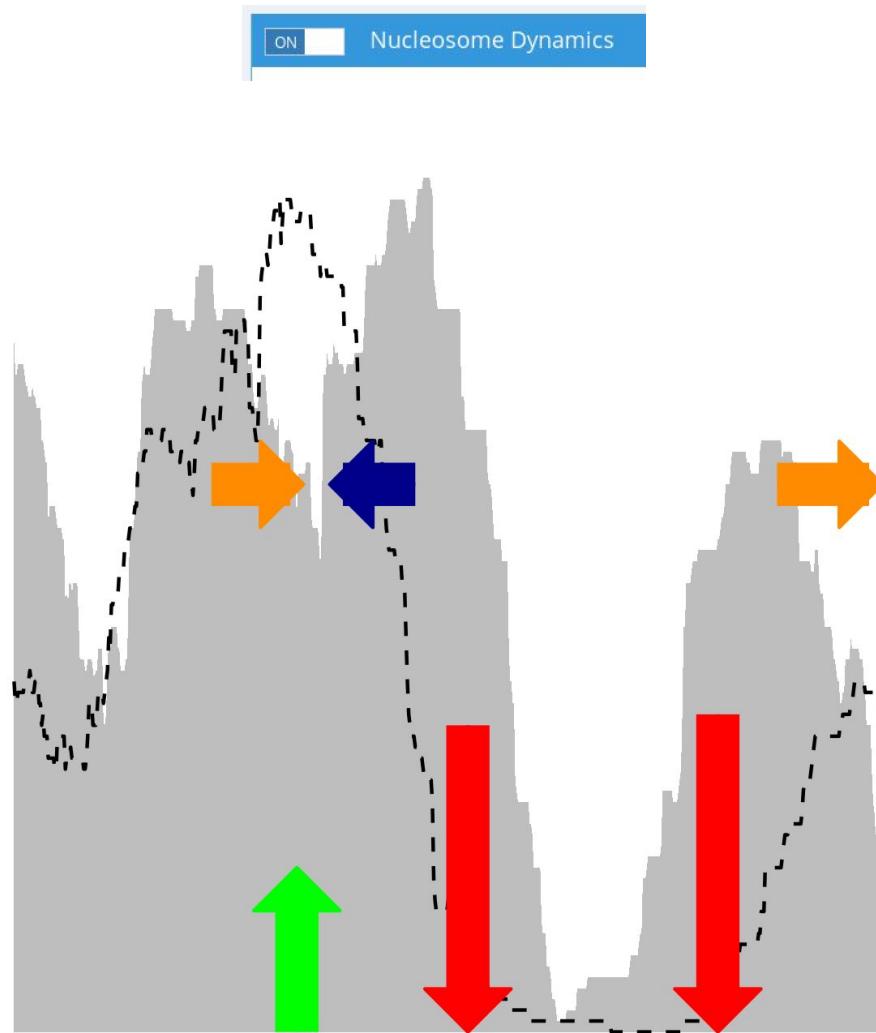


# Whole genome table

	A	B	C	D	E	F	G	H	I	J	K
1	Name	Total Nucleosomes	Total Well-Positioned	Total Fuzzy	Total Uncertain	TSS class	Distance from -1 to +1	Score phase	Score autocorrelation	Mean_STF	StdDev_STF
2	YBL001C	3	2	1	0	W-open-W	256	16	0.369608596978074	0.164088029561699	0.0387147909340138
3	YBL002W	6	2	4	0	-1 missing	NA	74	0.349978564457133	0.141989740127369	0.0696933074971864
4	YBL003C	6	1	5	0	W-close-W	202	55	0.446749087659194	0.124750201169941	0.051216456302949
5	YBL004W	56	7	45	4	W-open-W	321	22	0.830899396123374	0.0919428405158479	0.029590568441744
6	YBL005W	25	6	19	0	F-close-W	169	68	0.717834849072418	0.109605656861766	0.0339710831530526
7	YBL006C	5	3	2	0	F-close-W	133	42	0.537448426849847	0.128400897160577	0.0567264334183441
8	YBL007C	24	12	12	0	W-open-W	277	32	0.80853710999272	0.09705299159773	0.0263645037593939
9	YBL008W	17	8	8	1	W-open-W	222	58	0.834098517759126	0.118458082044361	0.0452103955786102
10	YBL009W	18	9	9	0	W-open-W	304	29	0.781164815691985	0.140062218731062	0.113667519178991
11	YBL010C	6	5	1	0	W-open-W	304	3	0.754891573823907	0.151377635519592	0.0548998015160063
12	YBL011W	20	7	13	0	F-overlap-F	75	15	0.770883933798821	0.105504612597916	0.0359769001359127
13	YBL014C	22	7	14	1	W-open-W	318	18	0.747931719819917	0.121833637607324	0.10745996983767
14	YBL015W	14	7	7	0	W-close-F	181	43	0.85532671133163	0.099868043707128	0.0331180240724269
15	YBL016W	8	8	0	0	W-close-W	150	22	0.745902911199523	0.127271896098687	0.0424689030467159
16	YBL017C	37	14	23	0	W-open-W	271	8	0.841200616717385	0.122294800355993	0.0932990724107503
17	YBL018C	4	3	1	0	W-open-W	276	17	0.600663700036704	0.171769777988211	0.0748153873428586
18	YBL019W	13	8	5	0	F-close-W	192	54	0.745863529717833	0.142637167354673	0.103822004527861
19	YBL020W	14	10	4	0	W-open-W	280	1	0.819574898980984	0.129943655959878	0.0511752808380884
20	YBL021C	4	4	0	0	W-open-W	280	48	0.359784718007489	0.149492650479416	0.0888991286111514
21	YBL022C	25	11	14	0	W-open-W	256	41	0.807806276783588	0.107242356457622	0.0502573207549877
22	YBL023C	22	7	15	0	F-open-W	302	60	0.845195094388813	0.0992661928178788	0.0272568004845798
23	YBL024W	15	8	7	0	W-open-W	313	38	0.818641894531484	0.125636148114694	0.0972395749321252
24	YBL025W	6	6	0	0	W-open-W	244	55	0.677065996415118	0.118380455652527	0.0323277293298724
25	YBL026W	5	4	1	0	W-open-W	258	69	0.422239576532508	0.164720022599566	0.09642073176602
26	YBL027W	7	2	5	0	F-close-W	204	79	0.739378782721932	0.107696606792407	0.036840130206131
27	YBL028C	4	3	1	0	-1 missing	NA	49	0.420180426048063	0.222970114539496	0.169530526749647
28	YBL029C-A	3	3	0	0	F-open-W	280	50	0.134116355032644	0.227231177545655	0.124581551486883
29	YBL029W	9	4	5	0	W-open-F	229	76.5	0.475979603453089	0.17696588211588	0.0731954768276163
30	YBL030C	8	4	4	0	F-close-F	165	45	0.814290237828308	0.105755014089908	0.0314631386086841
31	YBL031W	6	5	1	0	F-open-W	326	63.5	0.693241537128213	0.260103400526687	0.288136900040537



# With two BAMs as input: NucleosomeDynamics





# With two BAMs as input: NucleosomeDynamics

<input type="checkbox"/>	<input type="checkbox"/> uploads <small>i</small>		
<input checked="" type="checkbox"/>	cellcycleG2_chrlI.bam <small>i</small>	BAM	MNase-Seq
<input checked="" type="checkbox"/>	cellcycleM_chrlI.bam <small>i</small>	BAM	MNase-Seq

## MANAGE FILES

Actions ▼ Visualization ▼ Available Tools ▼

 [uploads / cellcycleG2\\_chrlI.bam i](#)  Nucleosome Dynamics

 [uploads / cellcycleM\\_chrlI.bam i](#) 

 [✖ Clear all files from list](#)



# NucleosomeDynamics' parameters

ON  Nucleosome Dynamics

Detection of local changes in the position of nucleosomes at the single read level observed between two reference nucleosome maps ([more information](#))

MNase-seq reference state (condition C1) [?](#)  
uploads / cellcycleG2\_chrlI.bam

MNase-seq final state (condition C2) [?](#)  
uploads / cellcycleM\_chrlI.bam

---

Genomic Range [?](#)  
All

Maximum Diff [?](#)  
70

Maximum Length [?](#)  
140

Shifts threshold [?](#)  
0.1

Shift minimum num. reads [?](#)  
3

Indels threshold [?](#)  
0.05

Indels minimum num. reads [?](#)  
3

---

Advanced Settings

Equal Size [?](#)  
 FALSE



## NucleosomeDynamics' parameters

**Maximum Diff:** maximum distance between fragment centers for them to be considered shifts

**Maximum Length:** Fragments longer than this value are removed in the preliminary filtering

**Equal Size:** Use this to trim all fragments to the same length

**Shift minimum num. reads:** Minimum number of reads in a shift hotspot

**Shift threshold:** Threshold for shift hotspots

**Indels minimum num. reads:** Minimum number of reads in an inclusion/eviction hotspot

**Indels threshold:** Threshold for inclusion/eviction hotspots



# NucleosomeDynamics's outputs

File	File type	Data type	Project	Date	Size	Actions
<input type="checkbox"/> ND	All	All	All			<input checked="" type="button"/> Clear filters
<input type="checkbox"/> ND_celcycleG2_chr... <small>?</small> BW	Nucleosome dyna...	sample-Nucleosom...	2018/09/16 19:45	2.63 M	<input type="button"/> <input type="button"/>	
<input type="checkbox"/> ND_celcycleG2_chr... <small>?</small> GFF3	Nucleosome dyna...	sample-Nucleosom...	2018/09/16 19:45	73.68 K	<input type="button"/> <input type="button"/>	

**bigWig output:**

-log10(pval) of the change significance across the genome

**GFF output:**

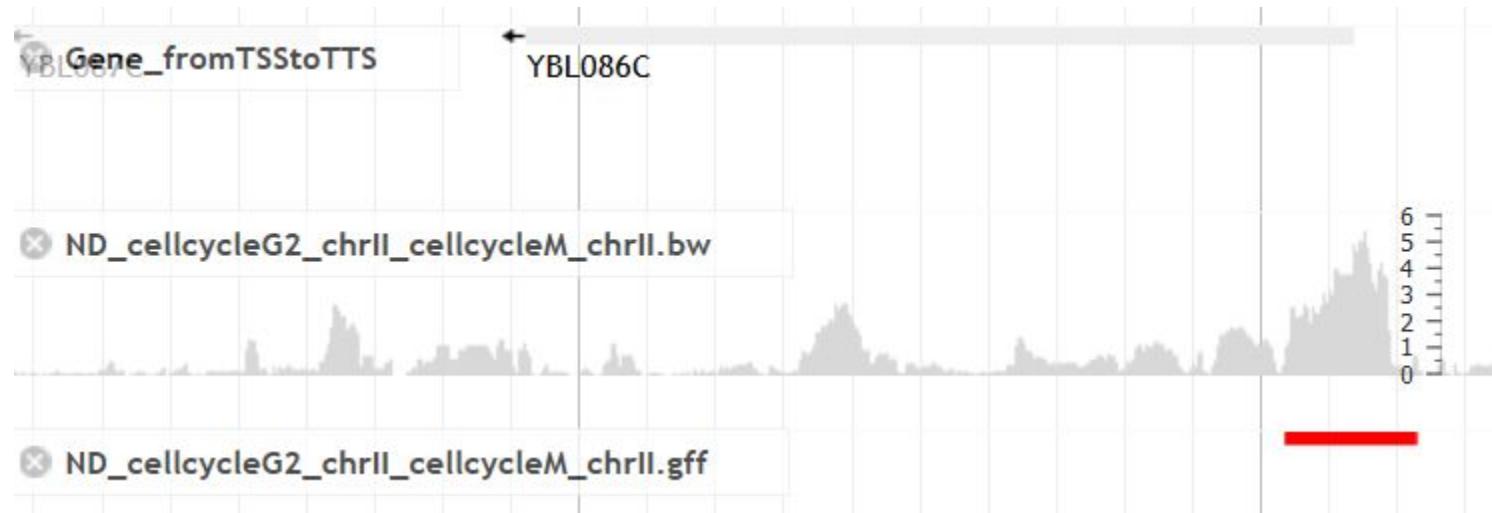
**score:** significance of the hotspot

**class:** SHIFT +, SHIFT -, EVICTION or INCLUSION

**nreads:** number of reads involved in the hotspot



# NucleosomeDynamics's outputs





# NucleosomeDynamics's outputs

Nucleosome change

**Primary Data**

Type ? Nucleosome change  
[Click to view a detailed plot 1000bp around the change]

Score ? 0.0202413713004625

Position ? chrII:62545..62788

Length 244 bp

Attributes

class ? EVICTION

nreads ? 3.12591941864113

source NucleosomeDynamics

**Region sequence**

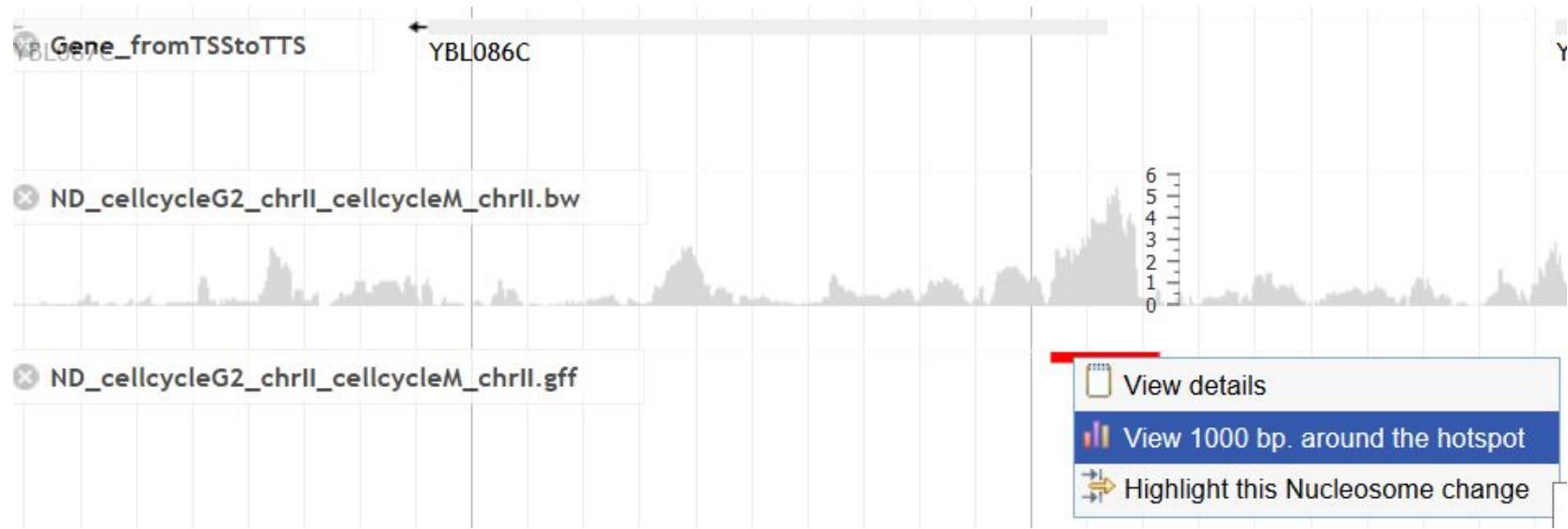
FASTA

```
>chrII chrII:62545..62788 class=Nucleosome change length=244
TTTGCAGGTCCAGTAGAAACTTGGCTTTGCCTTGCTATTATGATTAATGGCATCTTAAT
ATTTTGTGAGCGGTATTATACCTTCTTGGGTACCTAATACTTCTAACGCAACCTGAATGAAATT
AGAAGGTGTTCACTCCTTCATTAGCAAGTGAGCTTGCCGTTGTCACCGTACTGGTCATCTTA
AGAGATACAAAGGGGGGGGACCCTGAGAAAACCTCTCCGAAAAAAA
```

OK

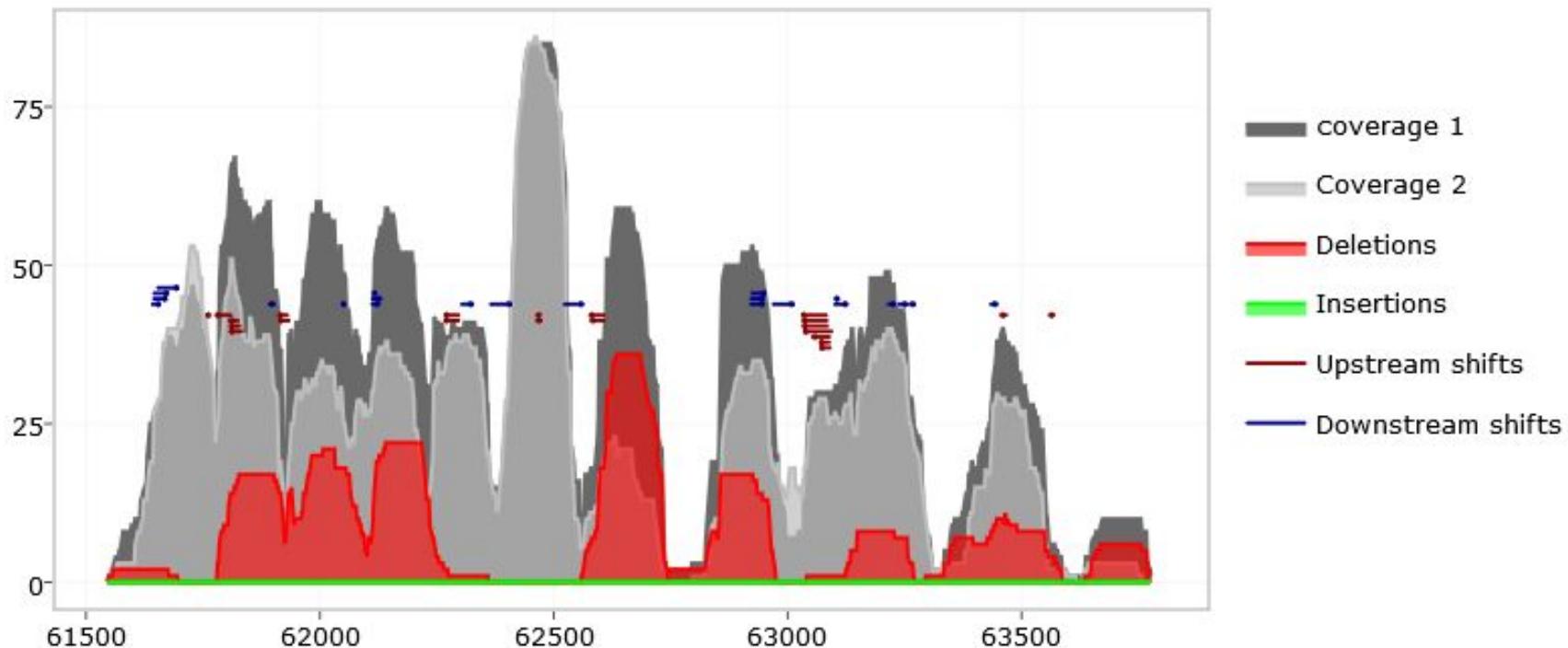


# Detailed interactive plot



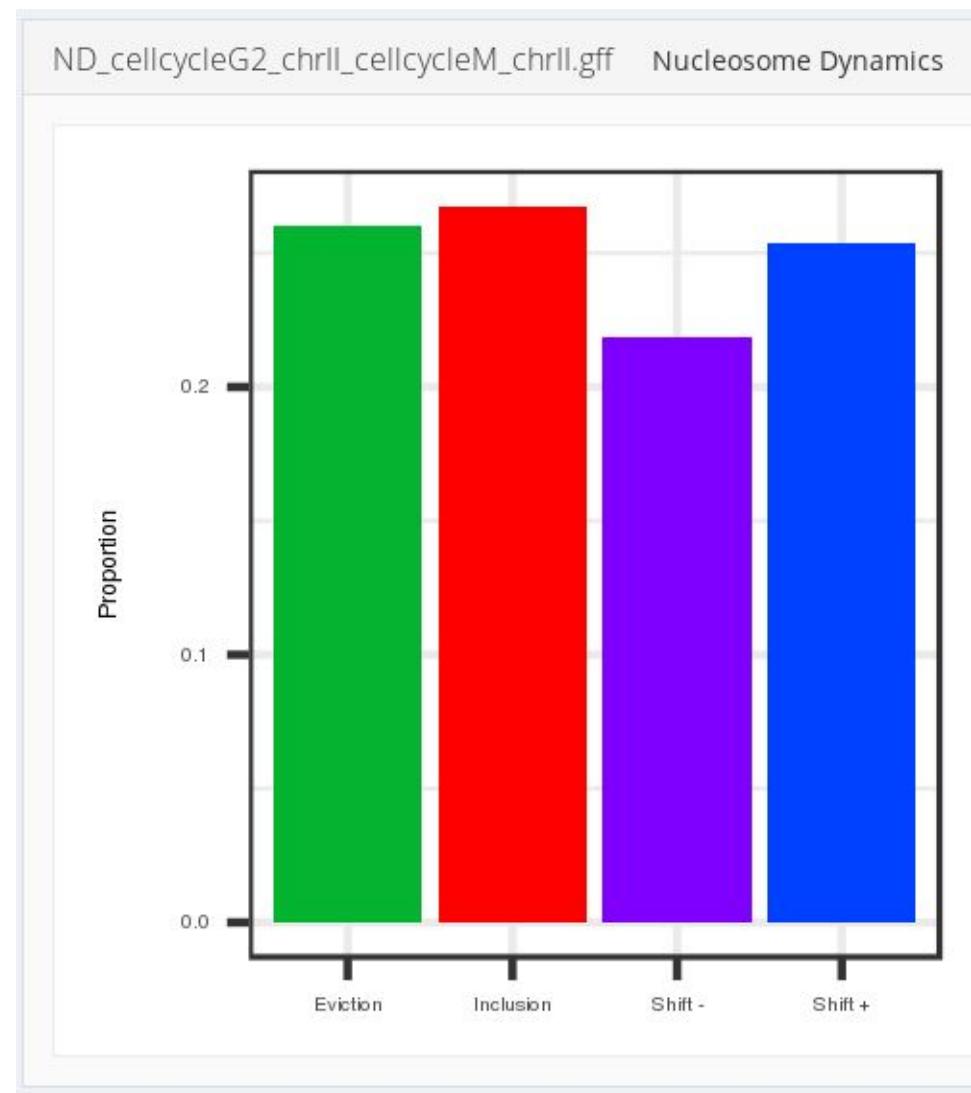


## Detailed interactive plot





# NucleosomeDynamics' statistics





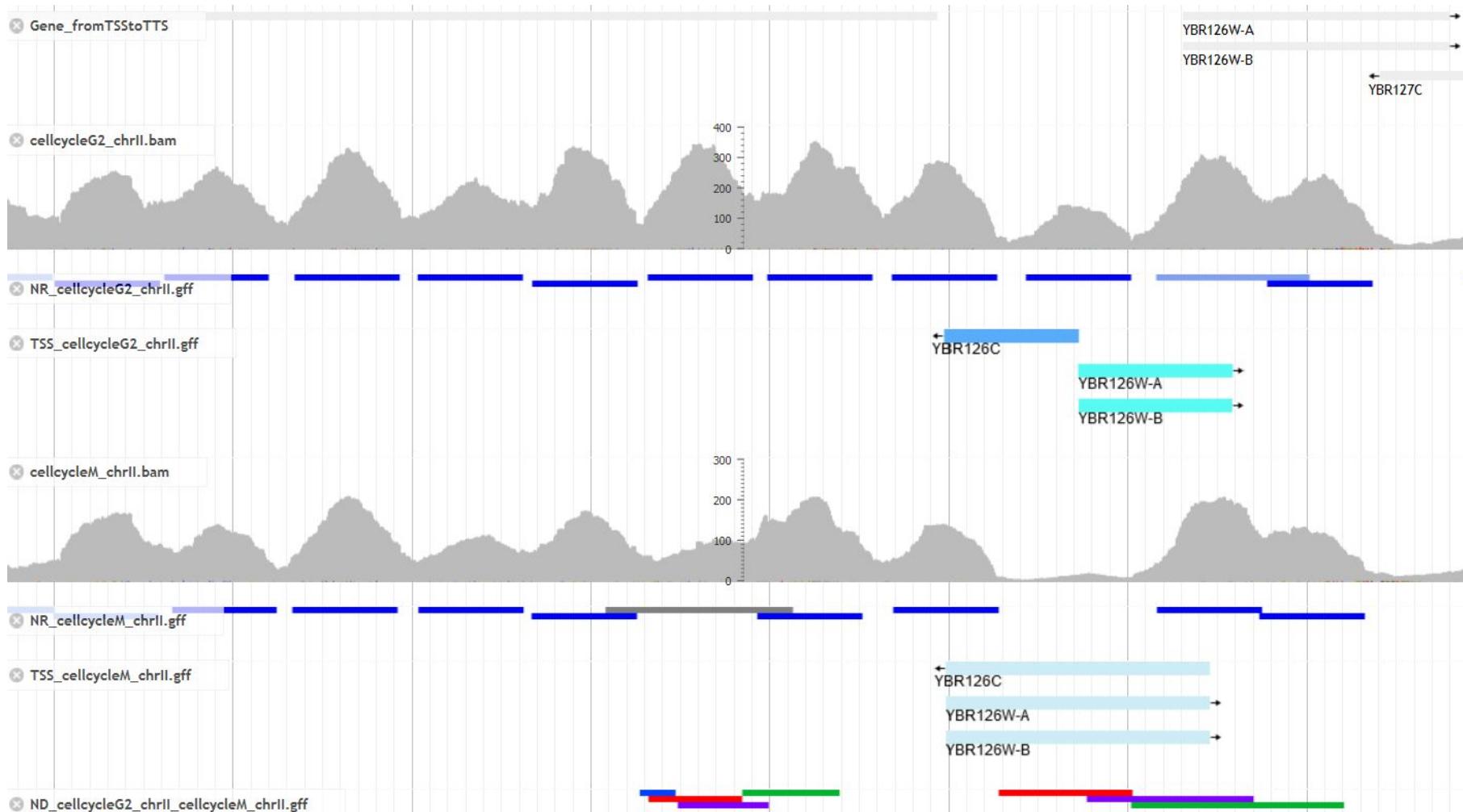
# Whole genome table

	A	B	C	D	E
1	Name	Inclusions	Evictions	Shifts+	Shifts-
2	YBL001C	1	0	0	1
3	YBL004W	0	1	1	0
4	YBL005W	1	0	1	1
5	YBL007C	1	0	0	1
6	YBL008W	1	1	1	0
7	YBL009W	0	2	1	1
8	YBL011W	2	0	1	2
9	YBL014C	1	2	2	2
10	YBL015W	1	0	2	0
11	YBL017C	3	2	1	3
12	YBL020W	1	0	0	0
13	YBL022C	1	0	2	0
14	YBL024W	1	0	1	0
15	YBL027W	0	1	0	0
16	YBL031W	1	0	0	1
17	YBL032W	1	0	0	0
18	YBL033C	1	0	1	1
19	YBL034C	0	2	0	0
20	YBL035C	1	0	0	0
21	YBL037W	1	0	1	1
22	YBL039C	0	0	1	0
23	YBL040C	0	1	0	0
24	YBL042C	0	0	1	0
25	YBL046W	2	0	1	2
26	YBL047C	2	1	0	1
27	YBL051C	0	0	1	0
28	YBL052C	0	1	0	0
29	YBL054W	0	1	1	1
30	YBL057C	1	0	0	0



# Visualizing it all together

We'll focus on chrII:489181..491246, promoter of the gene TPS1

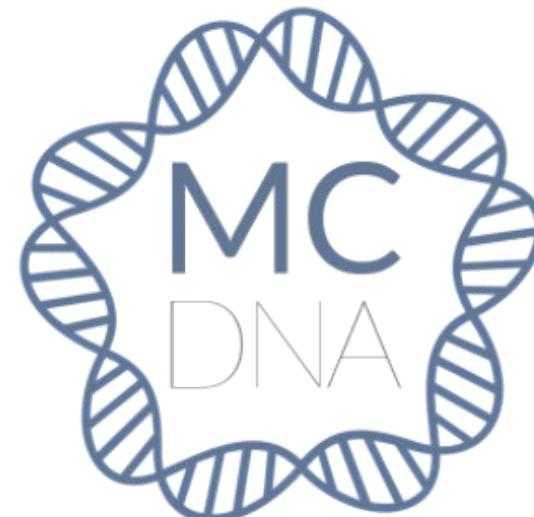




## Integration with other tools



Nucleosome Dynamics





Multiscale Complex Genomics

# Nucleosome Dynamics

A tool to analyze and visualize MNase-seq data



<http://www.multiscalegenomics.eu/>



@MuG\_genomics



<http://www.multiscalegenomics.eu/MuGVRE/mug-community/>



<http://www.multiscalegenomics.eu/MuG/contact/>



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