HARVARD T.H. CHAN
SCHOOL OF PUBLIC HEALTH

DANA-FARBER
CANCER INSTITUTE

Analyzing ChIP-Seq Data with SICER

Chongzhi Zang, PhD

Department of Biostatistics and Computational Biology
Center for Functional Cancer Epigenetics
Dana-Farber Cancer Institute
Harvard T.H. Chan School of Public Health

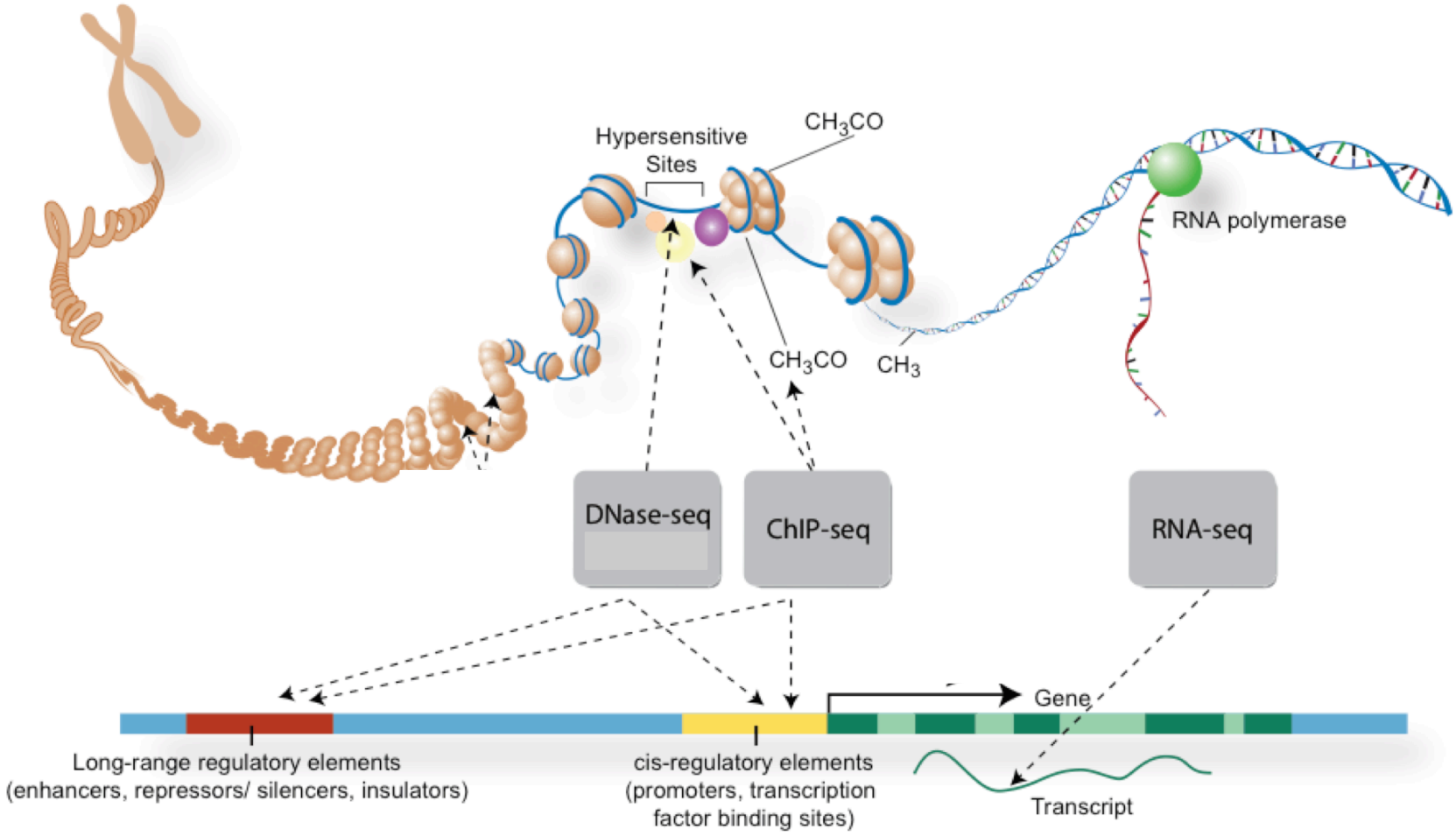
NCI BTEP Workshop on ChIP-seq Analysis
May 17, 2016

Outline

- ChIP-seq overview
- Characteristics of histone ChIP-seq data
- SICER algorithm
- Hands-on SICER tutorial

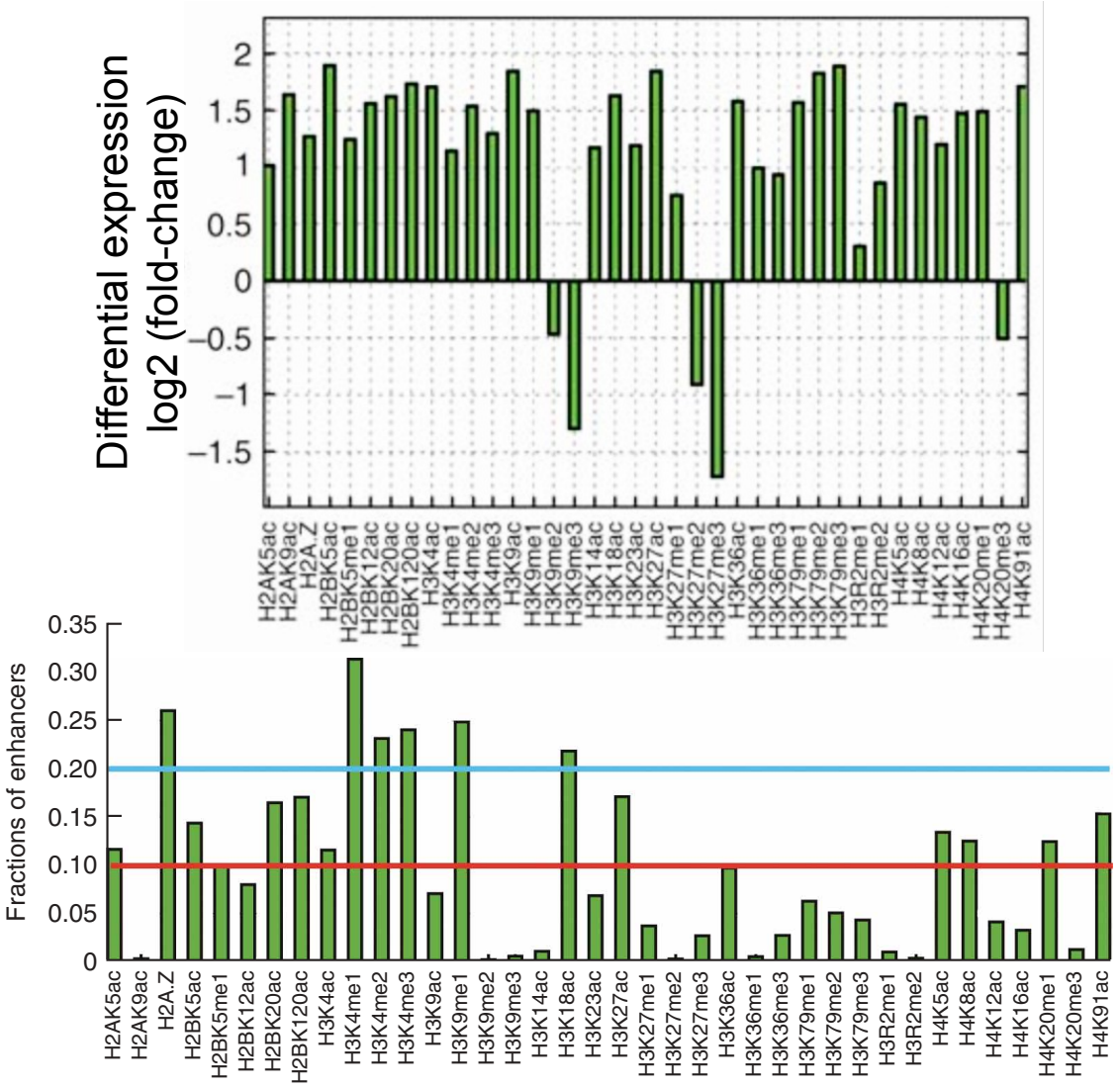
ChIP-seq overview

ChIP-seq is used to study the *in vivo* genome-wide location of a transcription factor or a histone modification



ENCODE Consortium

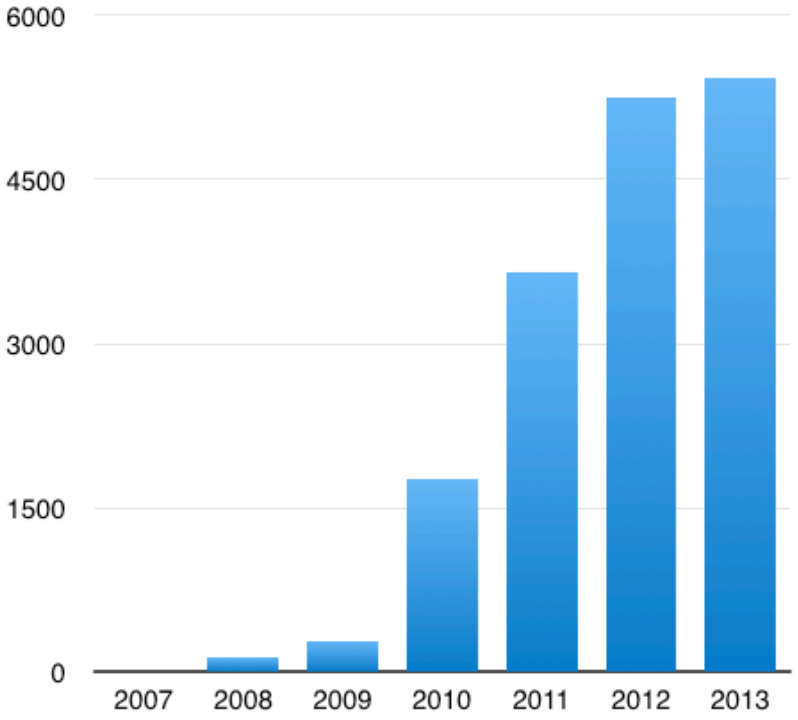
ChIP-seq profiles reveal gene regulatory functions of histone modifications

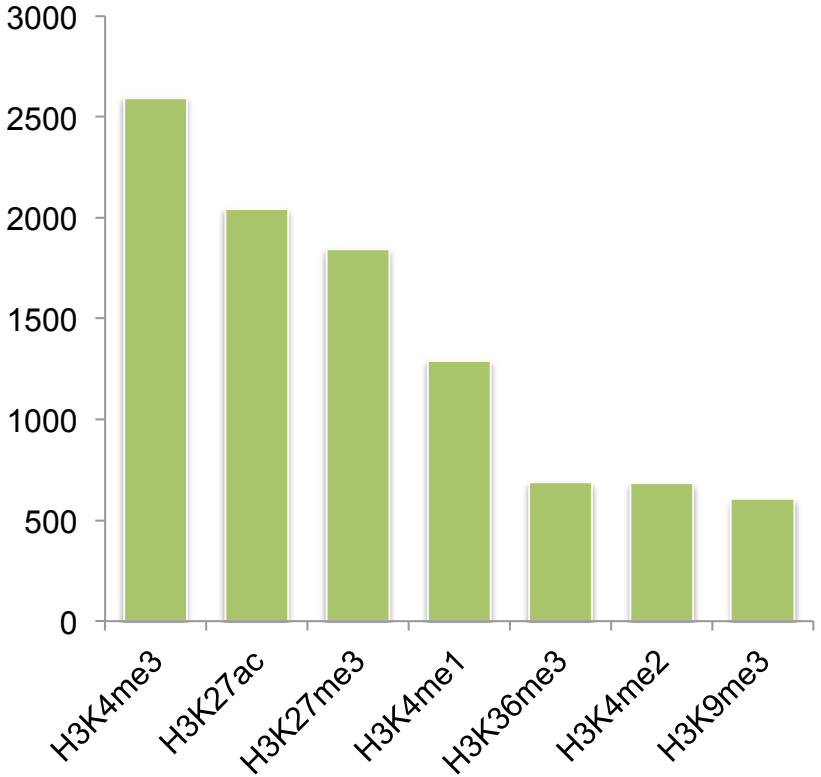


Wang, Zang et al. *Nat Genet* 2008

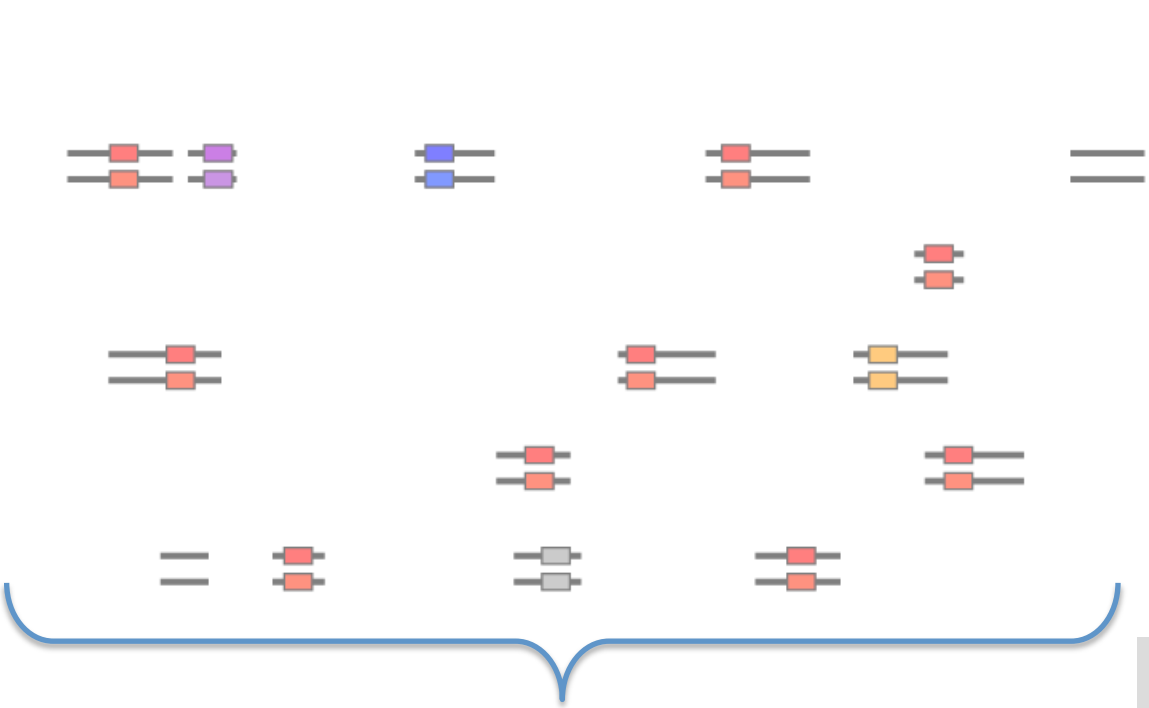
Public ChIP-seq data are skyrocketing
We are entering the “Big Data” era

Number of ChIP-seq datasets on GEO

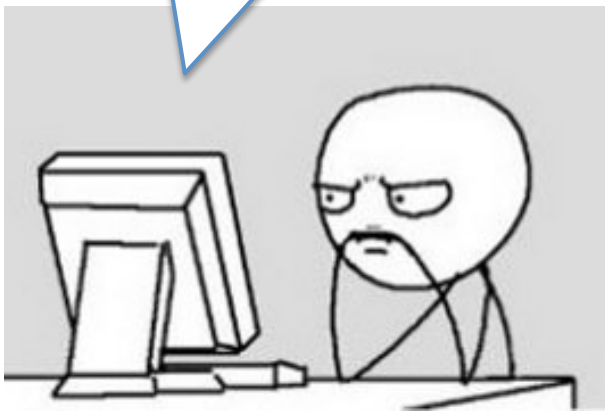




How ChIP-Seq is done



```
@ILLUMINA-8879DC:231:KK:3:1:1070:945 1:Y:0:
NNNAATACAGTCAGAAACATATCATATTGGAGAATA
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1153:945 1:Y:0:
NNNAAGCACACAGAAGATAACTAAACAATCAAGTAG
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1222:945 1:Y:0:
NNNAAGGGTCTTGAGAAGAAATCATTCTGGATGGCA
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1304:939 1:Y:0:
NNNCCAGGCTCCCGCGATTCTCCTGCCTCAGCTTCT
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1354:945 1:Y:0:
NNNCTCTTCCTTAGCTAAACTTTCAACTAAGCCAAA
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1411:932 1:Y:0:
NNNGTAGGACCATTGGCGTTGCGACACAAAAAATTT
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1496:937 1:Y:0:
NNNTTCATCGGGTTGAGAGTCCCCTTGTTGCATGCA
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1533:939 1:Y:0:
NNNATTTTCCCGTTCCAGGTCGCAATTTCCGCCGTT
#####################################
@ILLUMINA-8879DC:231:KK:3:1:1573:940 1:Y:0:
NNNGGGGTGCGCCTTTAGTCCCAGCTACTCAGGAAC
#####################################
```

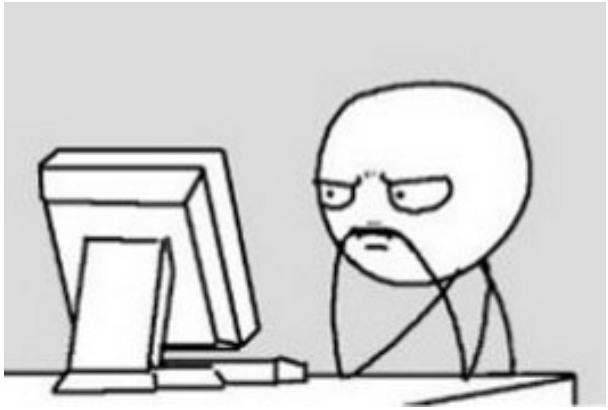


ChIP-seq data analysis

- Where in the genome do these sequence reads come from? - Sequence alignment and quality control

- What does the enrichment of sequence reads mean? - Peak calling (e.g. SICER, MACS)

- What can we learn from these data? – Downstream analysis and integration

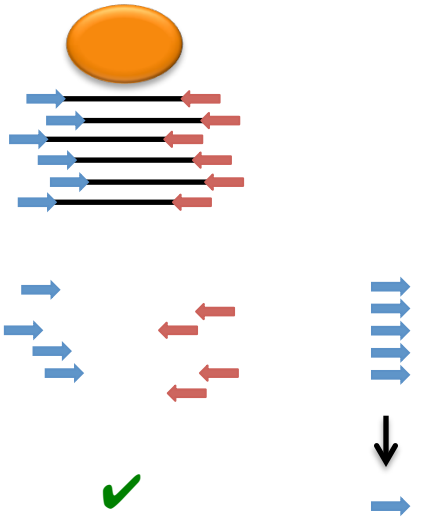


ChIP-Seq data analysis overview: basic processing

- alignment of each sequence read: **bowtie** or **BWA**

  cannot map to the reference genome ✗
  can map to multiple loci in the genome ✗
  can map to a unique location in the genome ✔

- redundancy control:

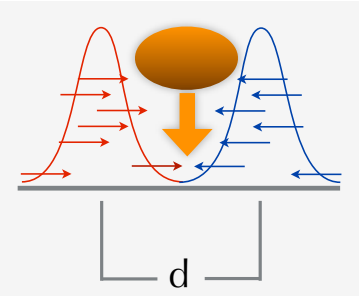


Langmead et al. 2009,
Zang et al. 2009

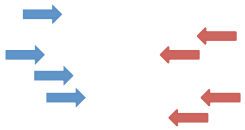
ChIP-Seq data analysis overview: basic processing

- DNA fragment size estimation

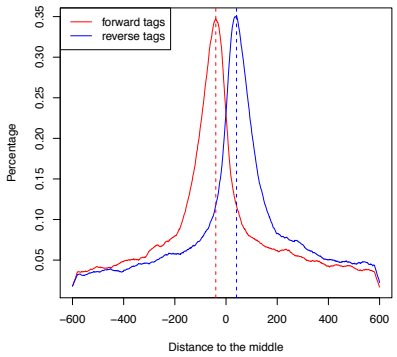
peak model

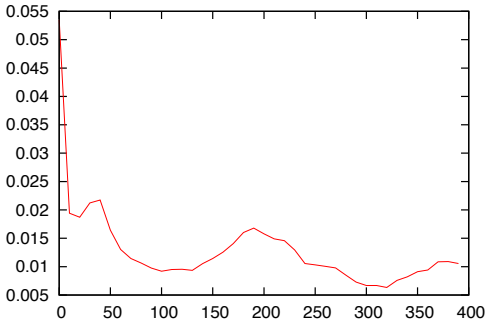


cross-correlation

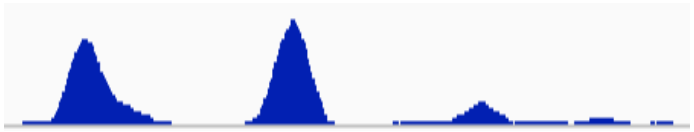


$$C(r) = \frac{1}{X}\int_x \left(T_+(x) - \overline{T_+}\right)\left(T_-(x+r) - \overline{T_-}\right)$$





- pile-up profiling



- Data visualization:
  - UCSC genome browser
  - IGV
  - WashU Browser

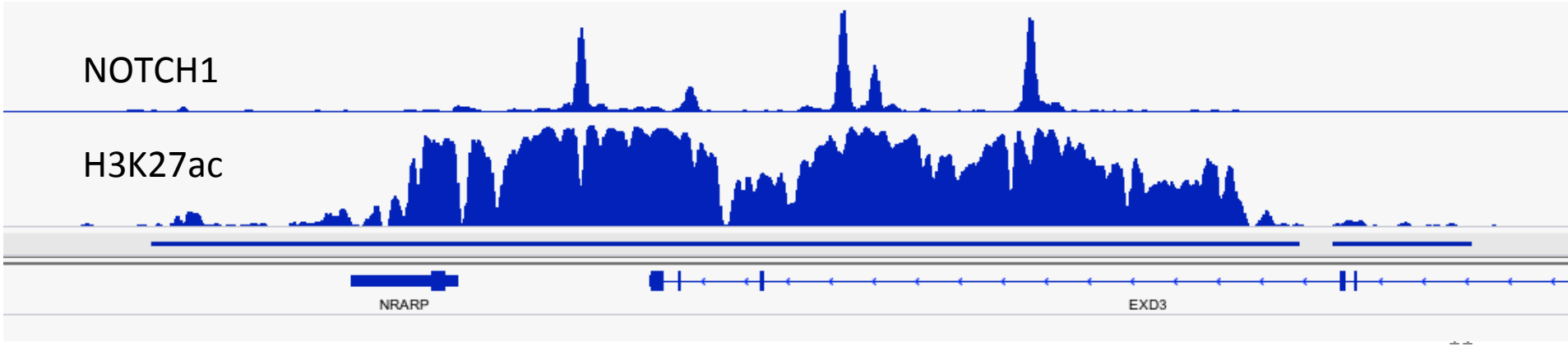
ChIP-Seq data analysis overview: peak calling

- **Sharp peaks**
  transcription factor binding, DNase HS

MACS (Zhang, 2008)

- **Broad peaks**
  histone modifications, “super-enhancers”
  Diffuse

SICER (Zang, 2009)
Spatial clustering of localized weak signal and integrative Poisson model



Wang, Zang et al. 2014

Characteristics of histone ChIP-seq data

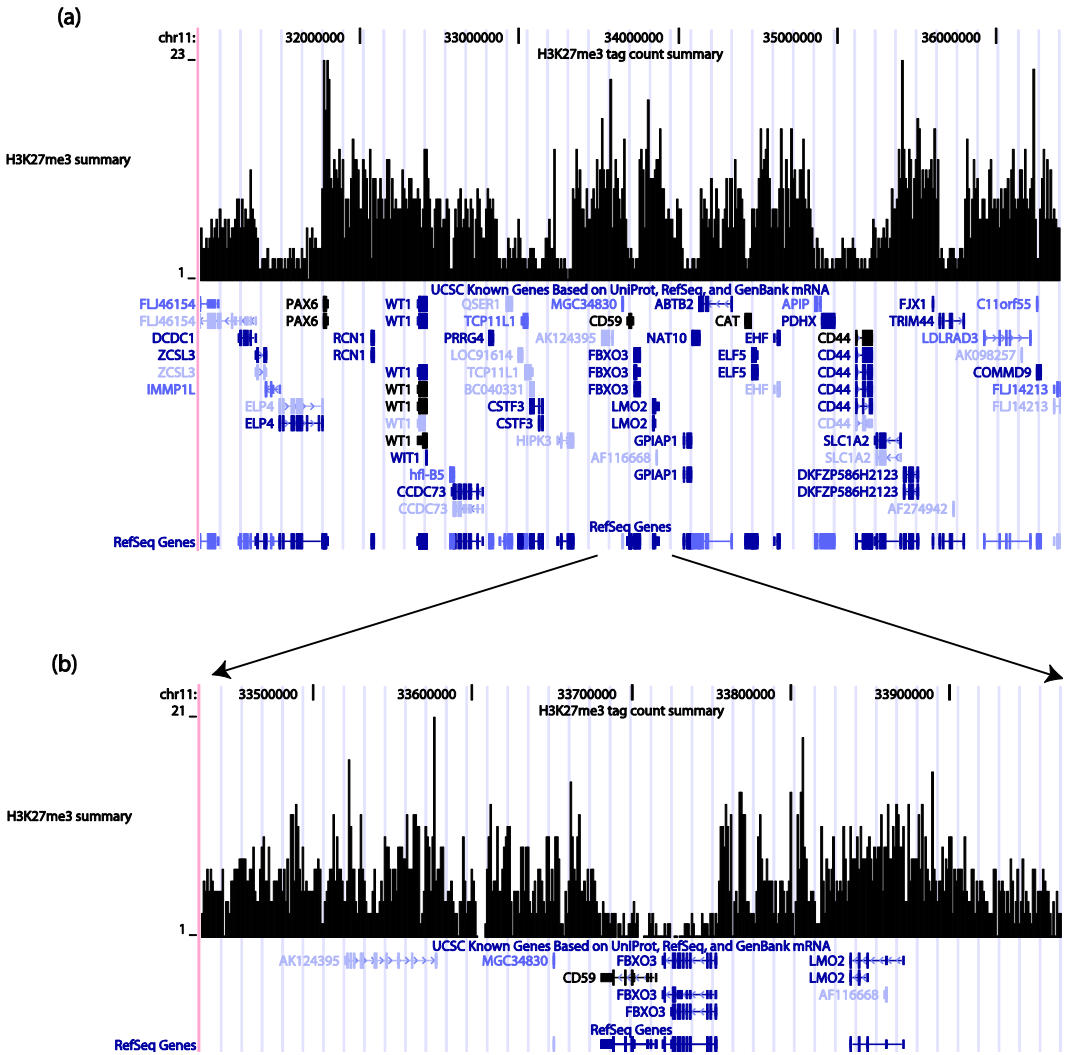
In other words, how to call “peaks” from such diffuse ChIP-seq data?

Histone modification patterns are diffuse

Characteristics:

- Noisy
- Unlike transcription factors
- Enriched regions are spread out
- Lack saturation

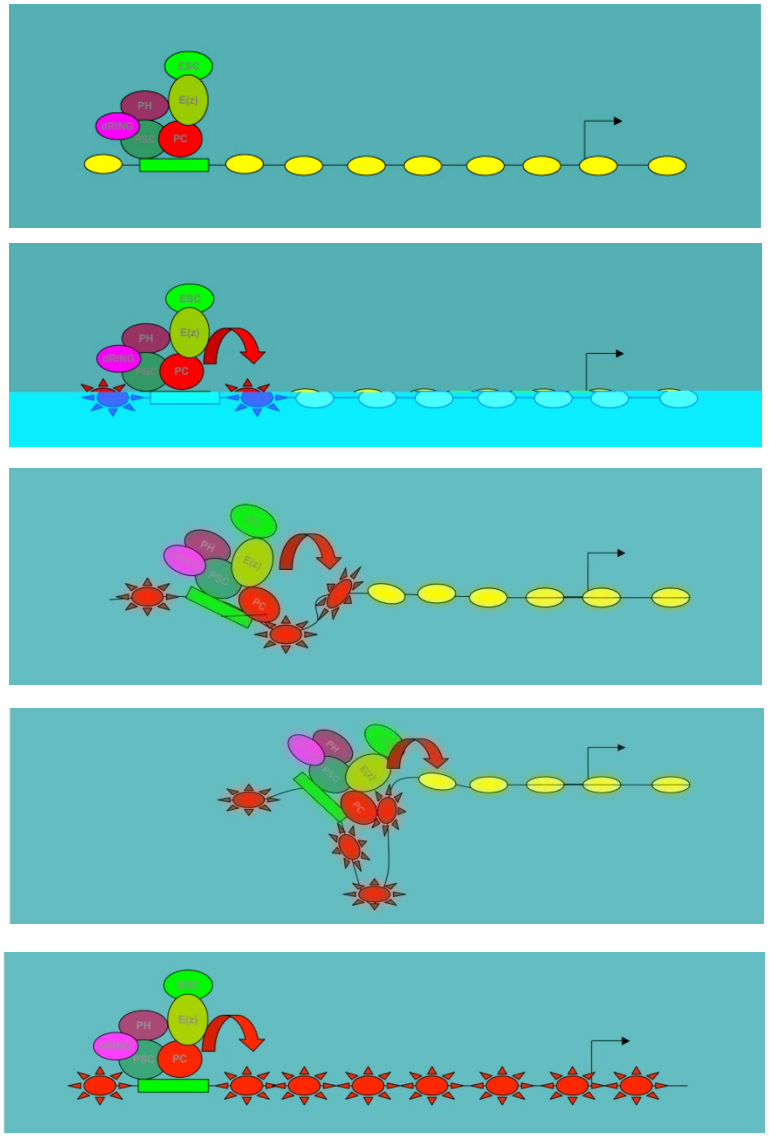
- *Why?*



Histone modification tends to spread out

Domain formation model for repressive marks

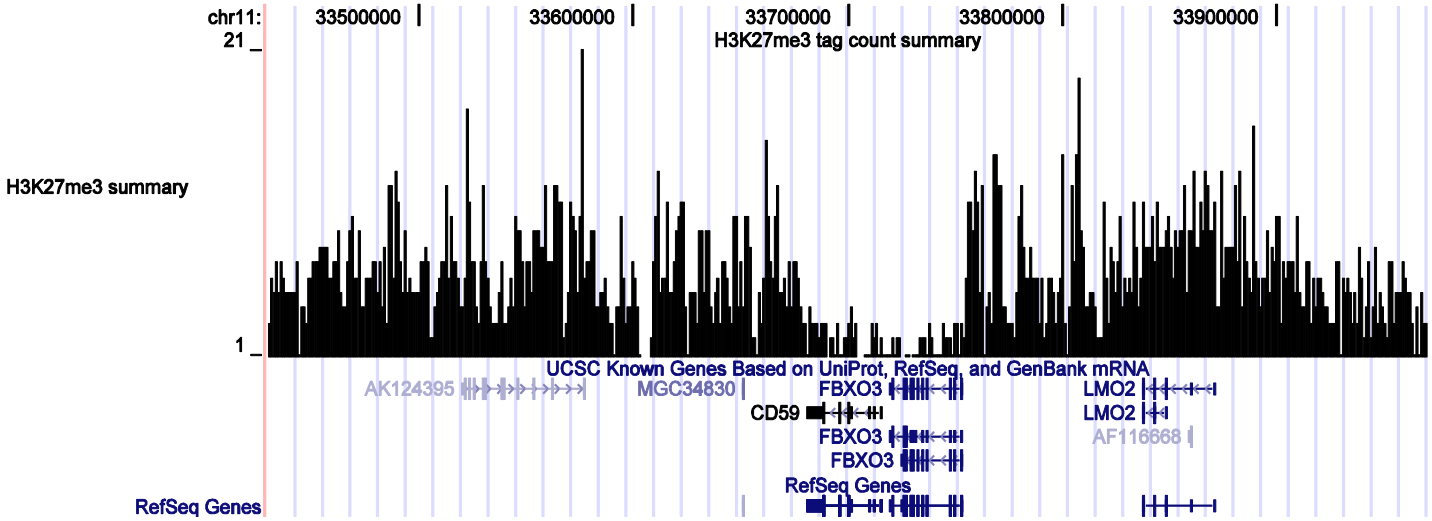
- Yeast:
  HP1
  H3K9me3
- Drosophila:
  PC1/PC2
  H3K27me3



Nucleation

Propagation

SICER: Motivation



- To detect broad/diffuse signals from ChIP-Seq
- Make use of the underlying biology
  - domain formation of histone modifications
- Account for background biases and provide statistical significance

SICER:

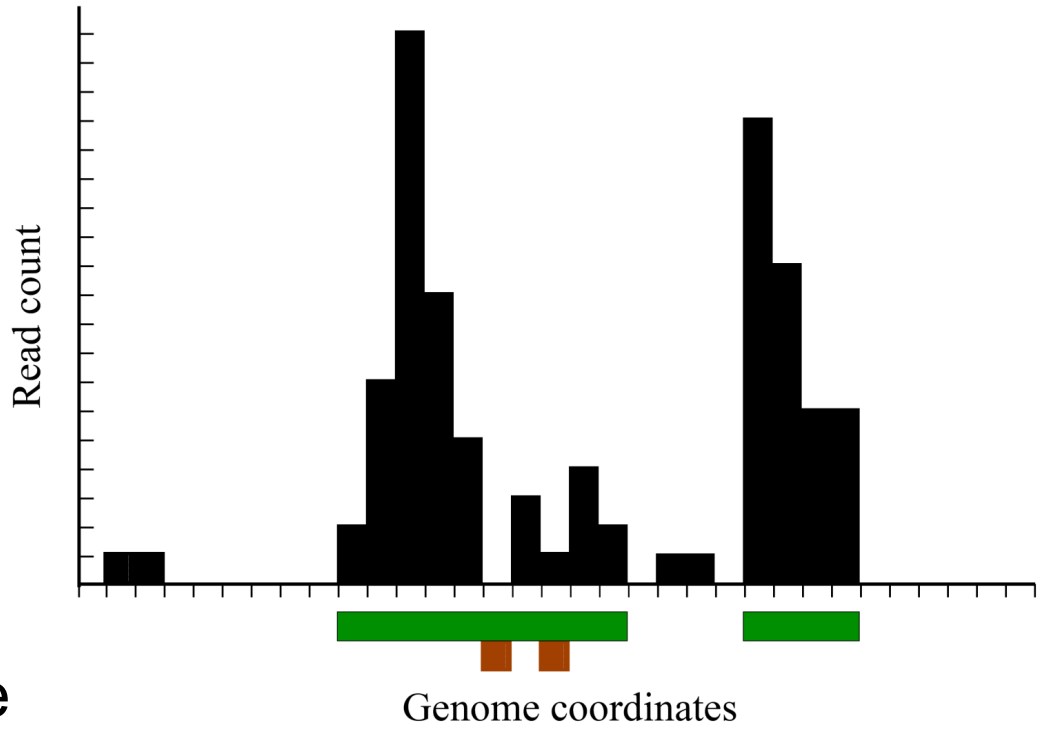
Spatial-clustering method for **I**dentification of **C**hIP-**E**nriched **R**egions

SICER: Definition of Island

- Eligible and ineligible windows

$$\sum_{l=l_0}^{\infty} P(l,\lambda) \leq p_0$$

- Eligible windows are separated by ***gaps*** of ineligible windows.
- **Island**: cluster of eligible windows separated by gaps of size at most *g* windows.



Example islands for $l_0 = 2$ and $g = 2$

Zang et al. *Bioinformatics* 2009

SICER: Scoring islands

- The scoring function is based on the probability of finding the observed tag count in a random background.
- For a window with ***m*** reads,
  - The probability of finding *m* reads is Poisson $P(m,\lambda)$
  - $\lambda = w\mathrm{N}/L$ is the average number of reads in each window
- Scoring function for an eligible window:

$$S = -\ln P(m,\lambda)$$

- Key quantity: the score of an island
  - Aggregate score of all eligible windows in the island
  - It corresponds to the background probability of finding the observed pattern

Zang et al. *Bioinformatics* 2009

SICER: Island score statistics

- Probability distribution of scores for a single window in a random background model:

$$\rho(s) = \sum_{l \geq l_0} \delta(s - s(l)) P(l, \lambda)$$

- Probability of a window being 'ineligible':

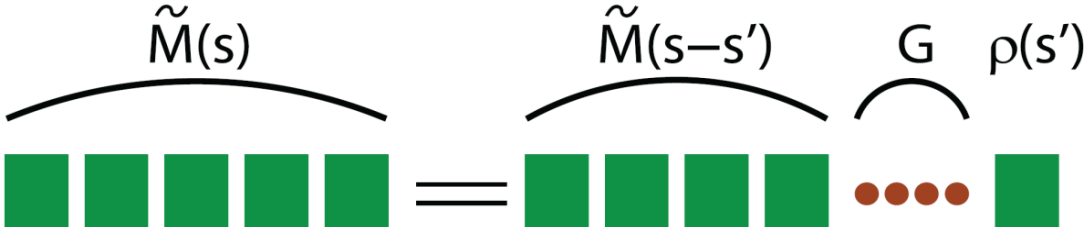
$$t = P(0, \lambda) + P(1, \lambda) + \cdots + P(l_0 - 1, \lambda)$$

- Gap factor:

$$G = 1 + t + t^2 + \cdots + t^g$$

Zang et al. *Bioinformatics* 2009

SICER: Island score statistics



- Recursion relation

$$\tilde{M}(s) = G(\lambda, l_0, g) \int_{s_0}^{s} \mathrm{d}s' \tilde{M}(s - s')\rho(s')$$

- Probability of finding an island of score ***s***:

$$M(s) = t^{g+1} \tilde{M}(s) t^{g+1}$$

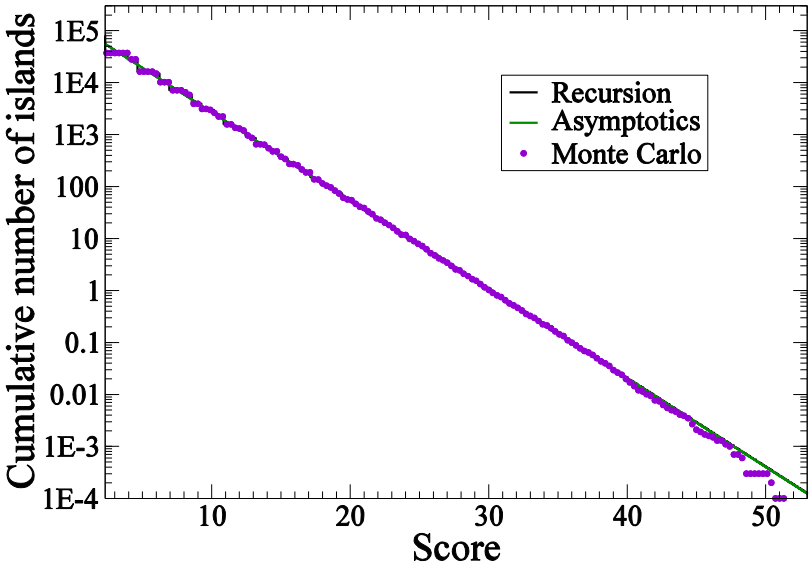
Zang et al. *Bioinformatics* 2009

SICER: Island score statistics

- Asymptotics of island score distribution in the random background

$$\tilde{M}(s) = \alpha \exp(-\beta s)$$

$$G(\lambda, l_0, g) \sum_{l \geq l_0} P(l, \lambda)^{1-\beta} = 1$$



- Statistic: *E*-value
  - Expected number of islands with score above s_T in the background

$$\sum_{s \geq s_T} LM(s) \leq e$$

Zang et al. *Bioinformatics* 2009

SICER: Significance determinations

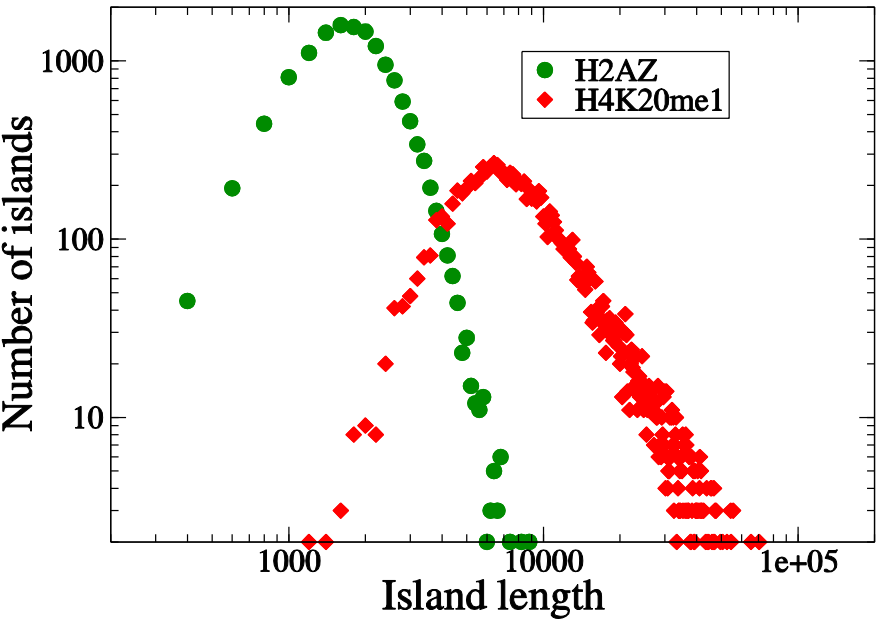
- Significance determination with random background model:
  - *E*-value determines an island score threshold

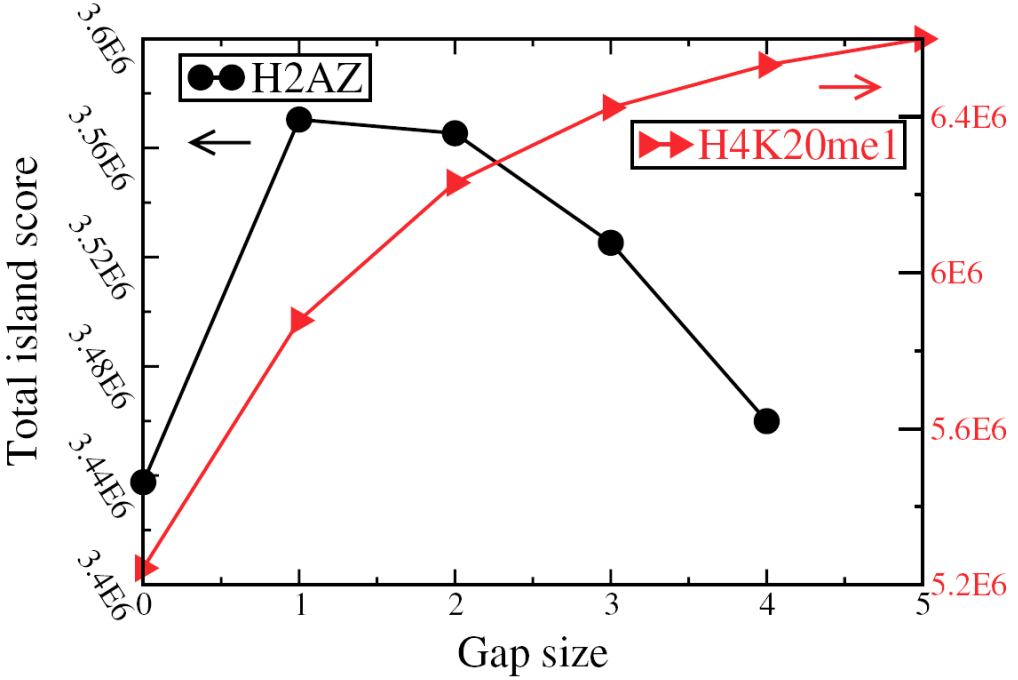
- Significance determination with control sample
  - Identify candidate islands using random background
  - For each candidate island, compare sample with control
  - *P*-value $\sum_{n=n_s}^{\infty} P(n_s, cn_c)$
  - False Discovery Rate (FDR)

Zang et al. *Bioinformatics* 2009

SICER: Choosing parameters

- Fragment size
- Window size: data resolution
- Gap size:



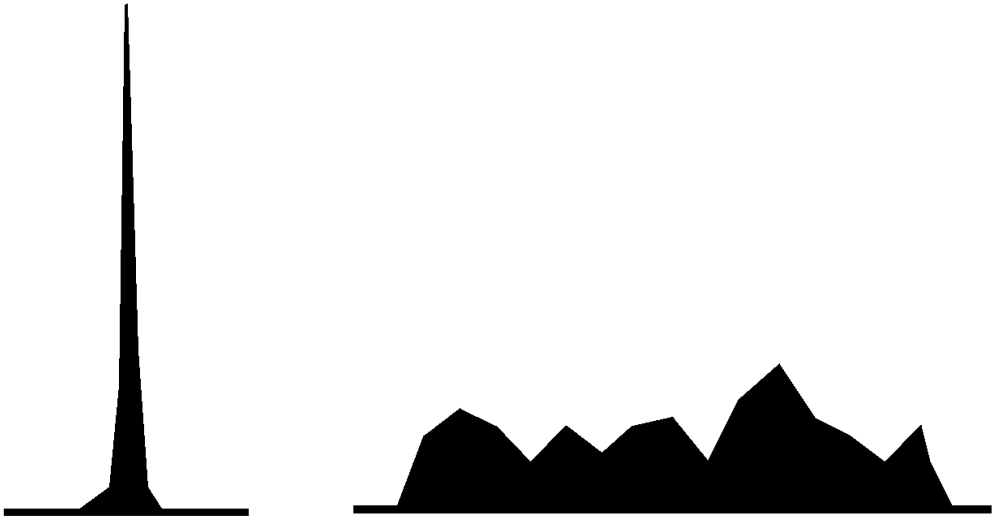


Zang et al. *Bioinformatics* 2009

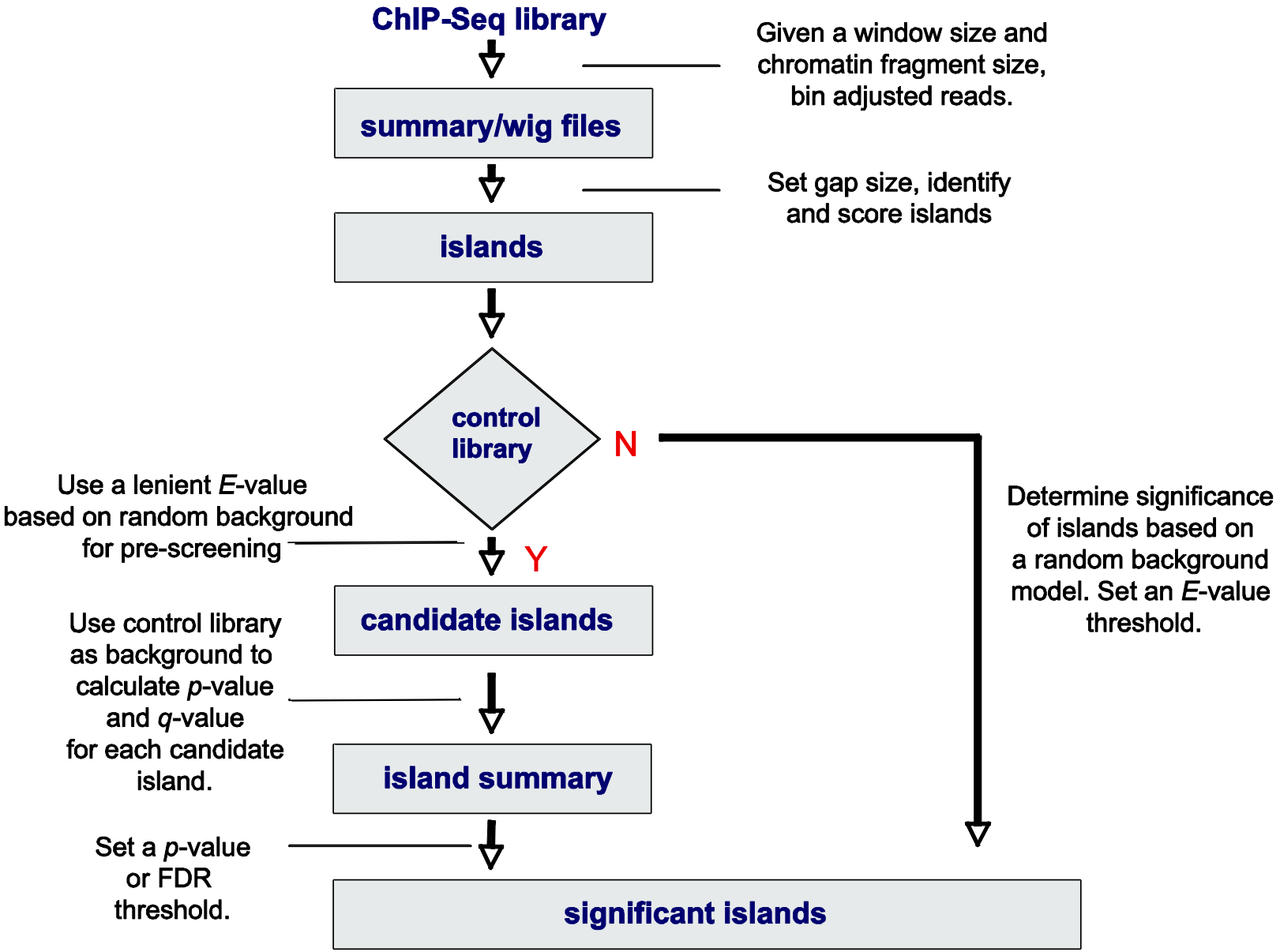
SICER: evaluation

- Compared with other methods, SICER focuses on the clustered enrichment rather than local enrichment.
- A schematic illustration:

- SICER can identify clustered enriched regions from diffuse data



SICER: Work flow



Zang et al. *Bioinformatics* 2009

SICER: Installation

- Download source code:

  http://home.gwu.edu/~wpeng/Software.htm

  Requirements: python and scipy (www.scipy.org)
- Galaxy

  https://usegalaxy.org/

- Genomatrix

ChIP-seq data examples

- http://cistrome.org/~czang/chipseqdata.htm

- Data format requirement:
  Mapped reads, BED format, 6 columns

chr11	10344210	10344260	255	0	-
chr4	76649430	76649480	255	0	+
chr3	77858754	77858804	255	0	+
chr16	62688333	62688383	255	0	+
chr22	33031123	33031173	255	0	-

  Mapped to reference genome: hg19, hg18, mm10, mm9, …
  BAMtools

Break

Install SICER, download test data

Run SICER

- Case study 1: without input control

  SICER-rb.sh

- Case study 2: with input control

  SICER.sh

- Case study 3: Differential calling

  SICER-df.sh

1. Run SICER without input control

- Data file: H3K27ac_act.bed
- Script: SICER-rb.sh
- Parameters:

["InputDir"]	..
["bed file"]	H3K27ac_act.bed
["OutputDir"]	.
["species"]	hg19
["redundancy threshold"]	1
["window size (bp)"]	200
["fragment size"]	150
["effective genome fraction"]	0.74
["gap size (bp)"]	600
["E-value"]	1000

Result output

Output file name	Description
H3K27ac_act-1-removed.bed	Non-redundant reads
H3K27ac_act-W200.graph	Raw data profile: bedGraph
H3K27ac_act-W200-normalized.wig	Raw data profile: wiggle
H3K27ac_act-W200-G600-E1000.scoreisland	Identified islands
H3K27ac_act-W200-G600-E1000-islandfiltered.bed	Island-filtered reads
H3K27ac_act-W200-G600-E1000-islandfiltered-normalized.wig	wiggle profile on identified islands

2. Run SICER with input control

- Data files: H3K27ac_act.bed and input_act.bed
- Script: SICER.sh
- Parameters:

[InputDir]	..
[bed file]	H3K27ac_act.bed
[control file]	input_act.bed
[OutputDir]	.
[Species]	hg19
[redundancy threshold]	1
[window size (bp)]	200
[fragment size]	150
[effective genome fraction]	0.74
[gap size (bp)]	600
[FDR]	0.01

Result output

Output file name	Description
H3K27ac_act-1-removed.bed	Non-redundant reads
H3K27ac_act-W200.graph	Raw data profile: bedGraph
H3K27ac_act-W200-normalized.wig	Raw data profile: wiggle
H3K27ac_act-W200-G600.scoreisland	Prescreened islands
H3K27ac_act-W200-G600-islands-summary	SICER summary
H3K27ac_act-W200-G600-islands-summary-FDR.01	SICER summary on identified islands
H3K27ac_act-W200-G600-FDR.01-island.bed	SICER identified islands
H3K27ac_act-W200-G600-FDR.01-islandfiltered.bed	Island-filtered reads
H3K27ac_act-W200-G600-FDR.01-islandfiltered-normalized.wig	wiggle profile on identified islands

3. Run SICER for differential peak calling

- Data files:
  H3K27ac_act.bed, input_act.bed
  H3K27ac_inh.bed, input_inh.bed
- Script: SICER-df.sh
- Parameters:

[KO bed file]	H3K27ac_act.bed
[KO control file]	input_act.bed
[WT bed file]	H3K27ac_inh.bed
[WT control file]	input_inh.bed
[window size (bp)]	200
[gap size (bp)]	150
[FDR for KO vs KOCONTROL or WT vs WTCONTROL]	0.01
[FDR for WT vs KO]	0.01

- What it does:
  1. Call peaks for “WT” and “KO” separately (SICER.sh)
  2. Identify union (merged) islands
  3. Compare “KO” vs. “WT” for increased islands
  4. Compare “WT” vs. “KO” for decreased islands

Output example

Output file name	Description
H3K27ac_act-vs-*H3K27ac_inh*-W200-G600-E-union.island	Merged islands
H3K27ac_act-and-*H3K27ac_inh*-W200-G600-summary	Merged island summary
H3K27ac_act-W200-G600-increased-islands-summary-FDR0.01	Identified increased islands
H3K27ac_act-W200-G600-decreased-islands-summary-FDR0.01	Identified decreased islands

Summary

- ChIP-seq for histone mark/epigenetic profiling
- ChIP-seq “broad peak” calling: SICER
- Use SICER for:
  - Peak calling: with or without input control
  - **Differential peak calling**
- SICER users group:

  https://groups.google.com/forum/#!forum/sicer-users

omictools.com

Acknowledgments

Weiqun Peng
Wenjing Yang

Keji Zhao
Dustin E. Schones
Zhibin Wang
Kairong Cui
Gang Wei
Tae-Young Roh
Artem Barski
Iouri Chepelev

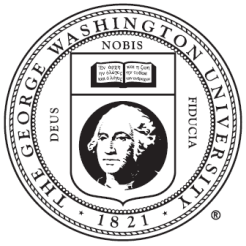
Chen Zeng

Xiaole Shirley Liu
Clifford Meyer
Tao Liu
Han Xu
Sheng'En Hu
Su Wang
Qian Qin
Sujun Chen

Gary Felsenfeld
Andre Nussenzweig
John O'Shea
Michael Q. Zhang
Nan-Ping Weng
Anand Swaroop

Myles Brown
Jun S Liu
Ramesh Shivdasani
Jon Aster
Warren Pear
Stephen Blacklow

All SICER users!



LEUKEMIA & LYMPHOMA SOCIETY®
fighting blood cancers

DANA-FARBER CANCER INSTITUTE

HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

Thank you very much!