Analysis of Next-Generation Sequencing Data for Epigenetics

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Outline

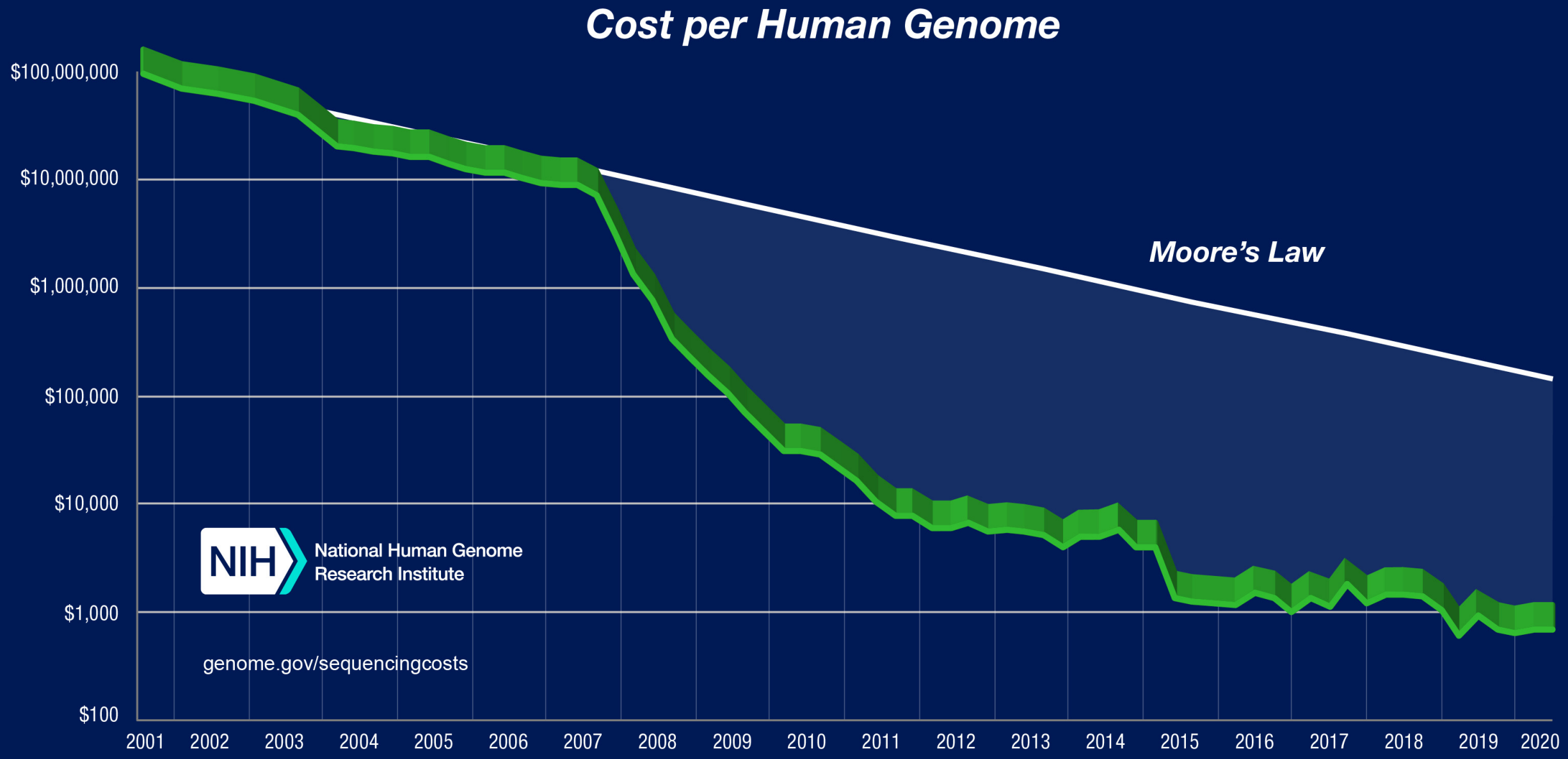
- 1st Half:
  - NGS introduction
  - NGS data analysis strategy
  - ChIP-seq data analysis

- 2nd Half:
  - Other NGS data analysis
  - Downstream analysis and integration
  - Online resources

Learning Objectives

- Understand how NGS works and key QC measures
- Learn how ChIP-seq data analysis is done
- Understand general strategies of NGS data analysis and online resources


Microhabitats save mammals, but not birds, from warming pp. 553 & 633
Gut microbiota modulate immunotherapy pp. 573, 595, & 602
Physically distanced quantum gates pp. 576 & 614
Science
$15
5 FEBRUARY 2021
sciencemag.org
AAAS
SPECIAL ISSUE
HUMAN GENOME AT
20


Cost per Human Genome
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
Moore's Law
NIH
National Human Genome
Research Institute
genome.gov/sequencingcosts
2001
2002
2003
2004
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2017
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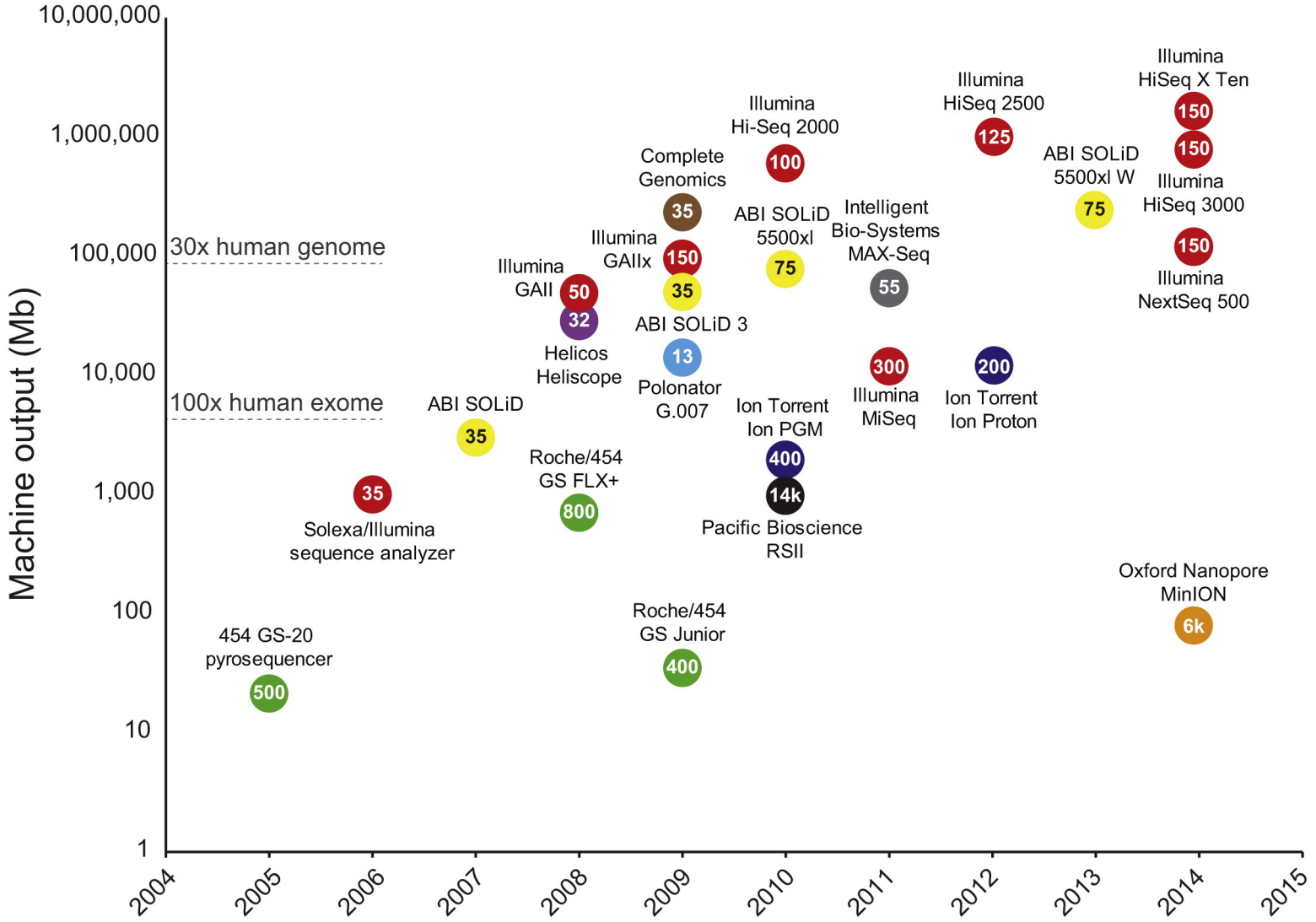
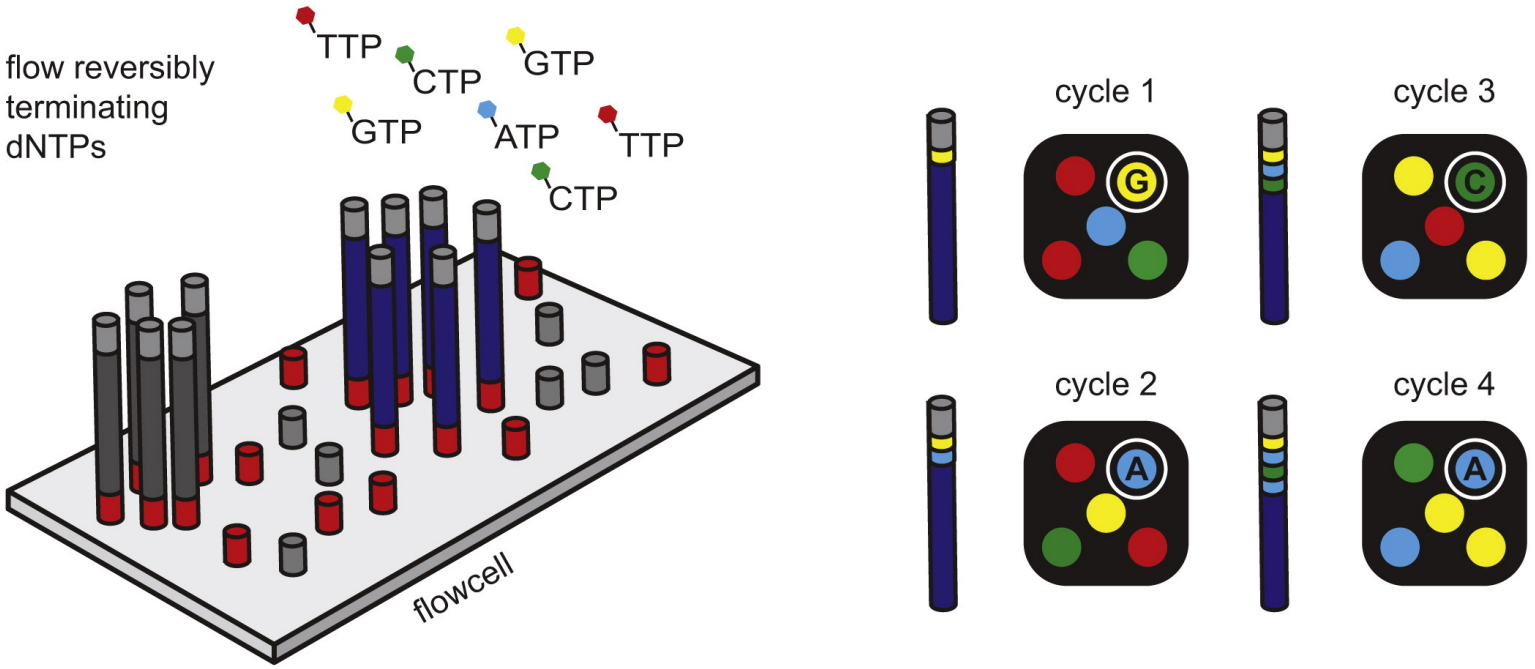
2019
2020

Development of commercial NGS instruments



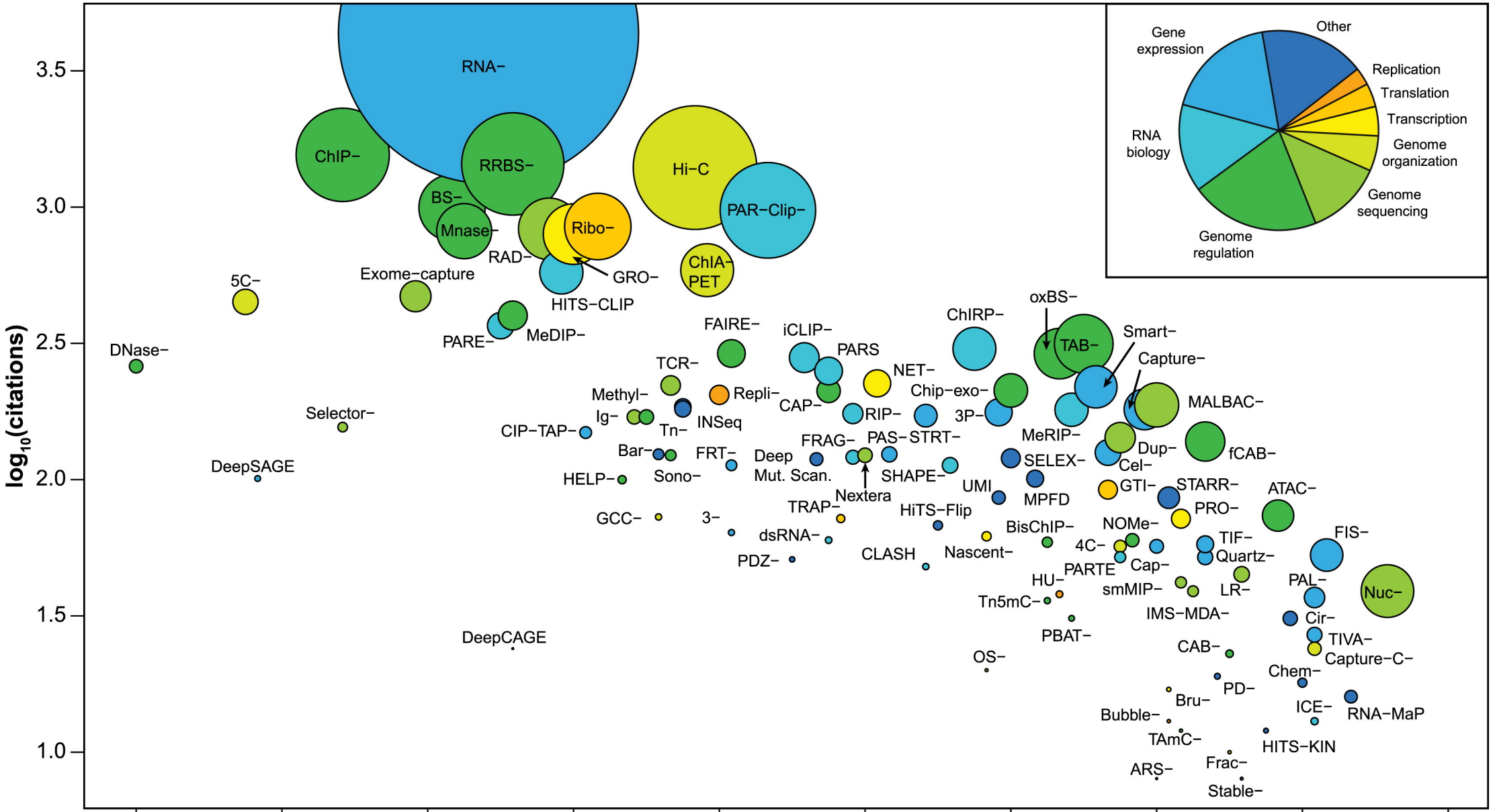
Reuter et al. Mol Cell 2015

Illumina/Solexa sequencing technology



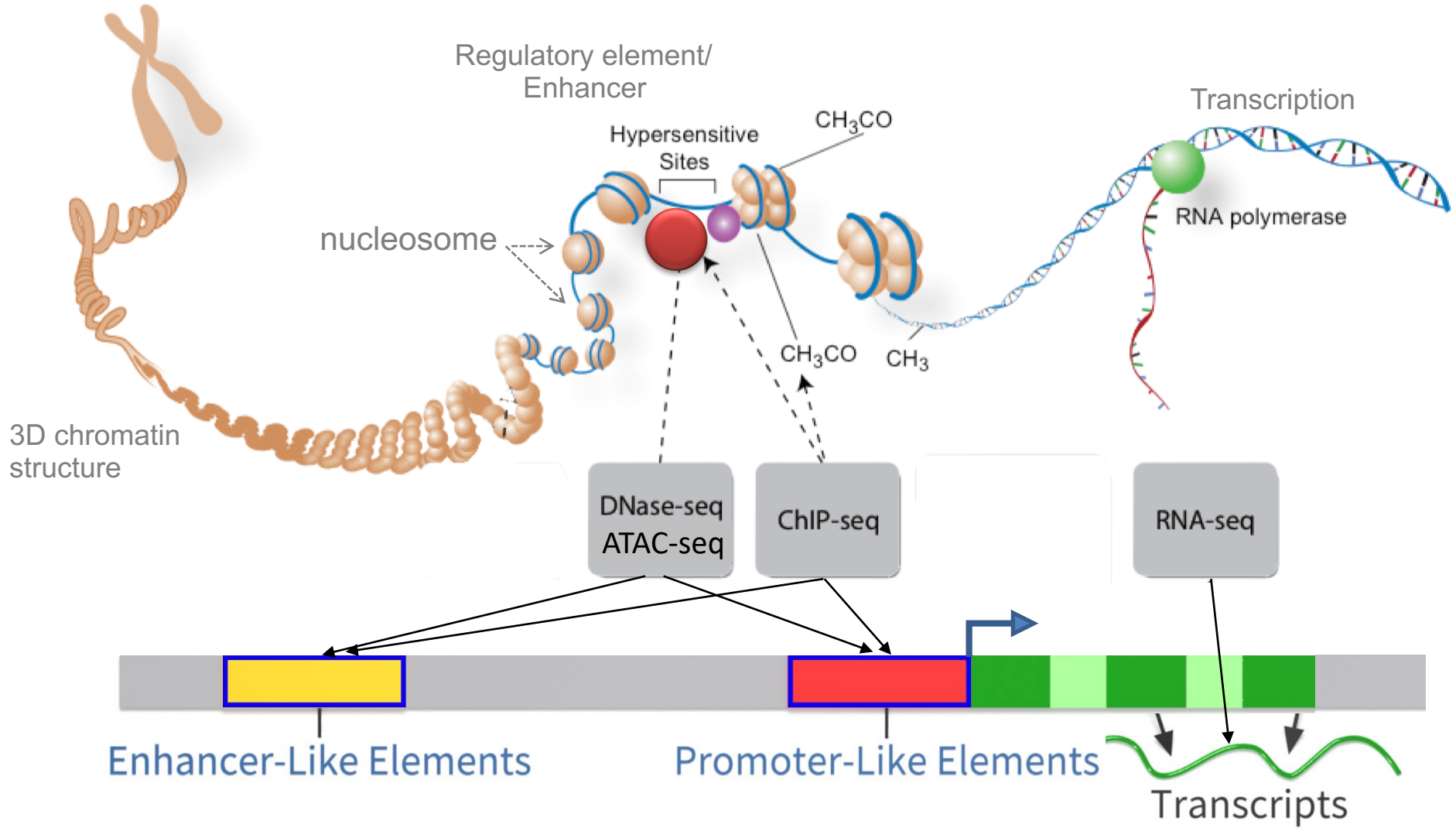
Reuter et al. Mol Cell 2015

NGS-based applications (-seq)

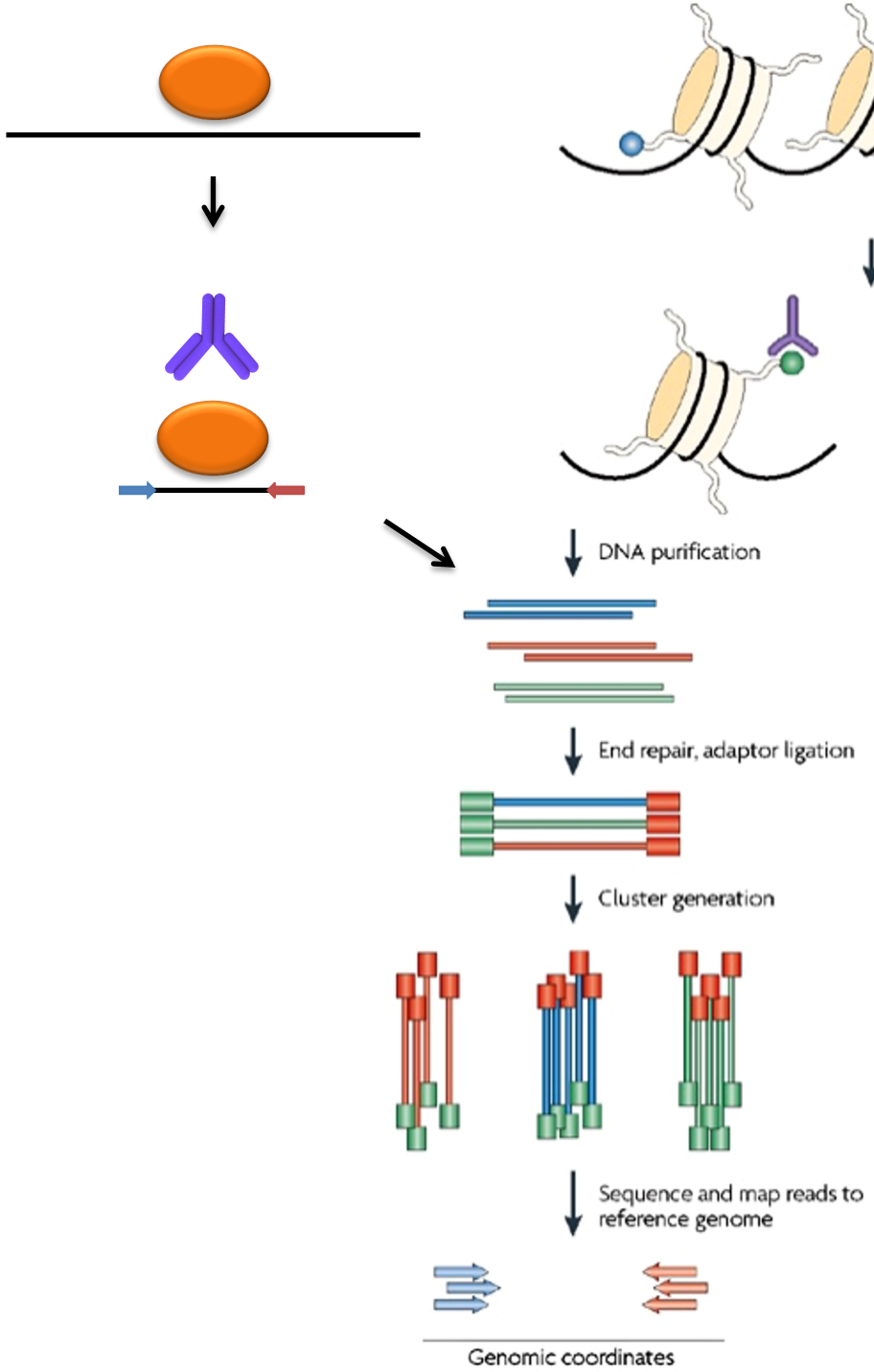


Reuter et al. Mol Cell 2015

NGS helps functional studies of the genome and epigenome



Modified figure from ENCODE, Darryl Leja (NHGRI), Ian Dunham (EBI)

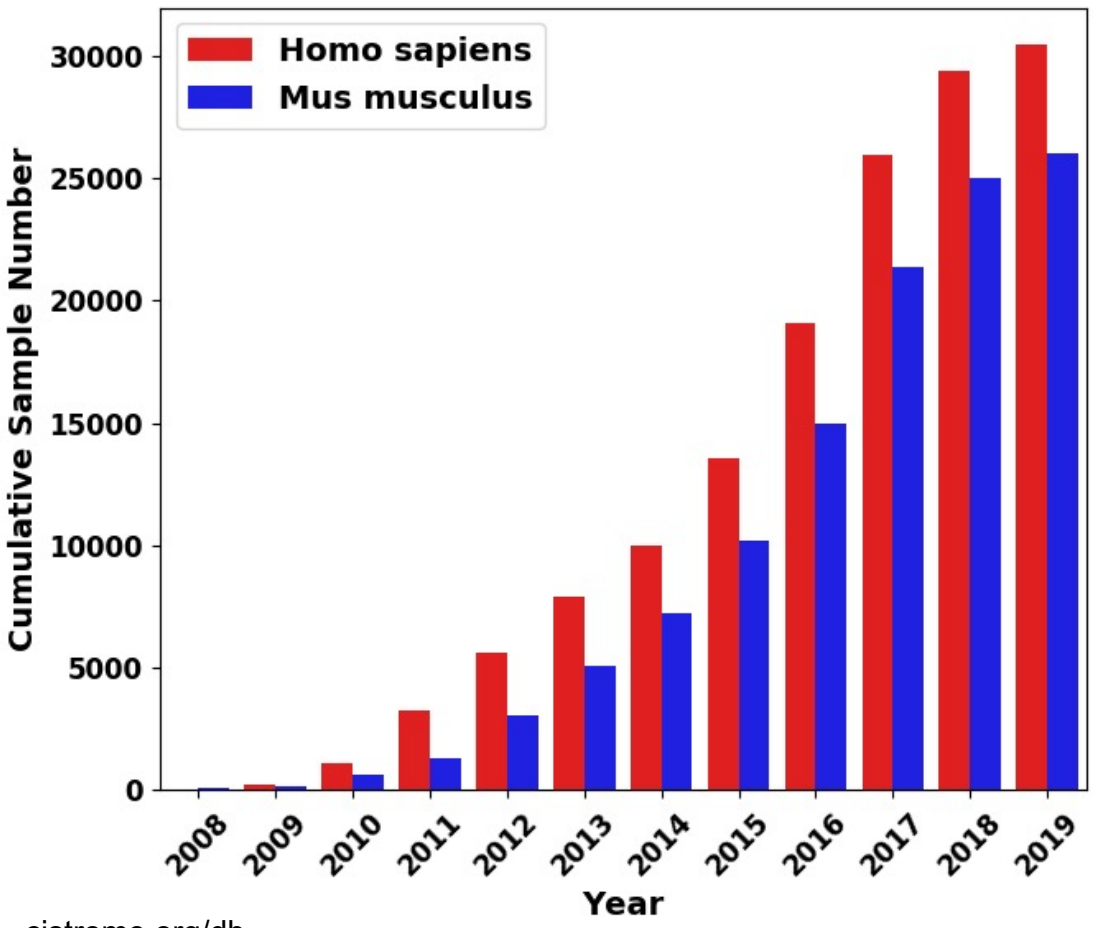


ChIP

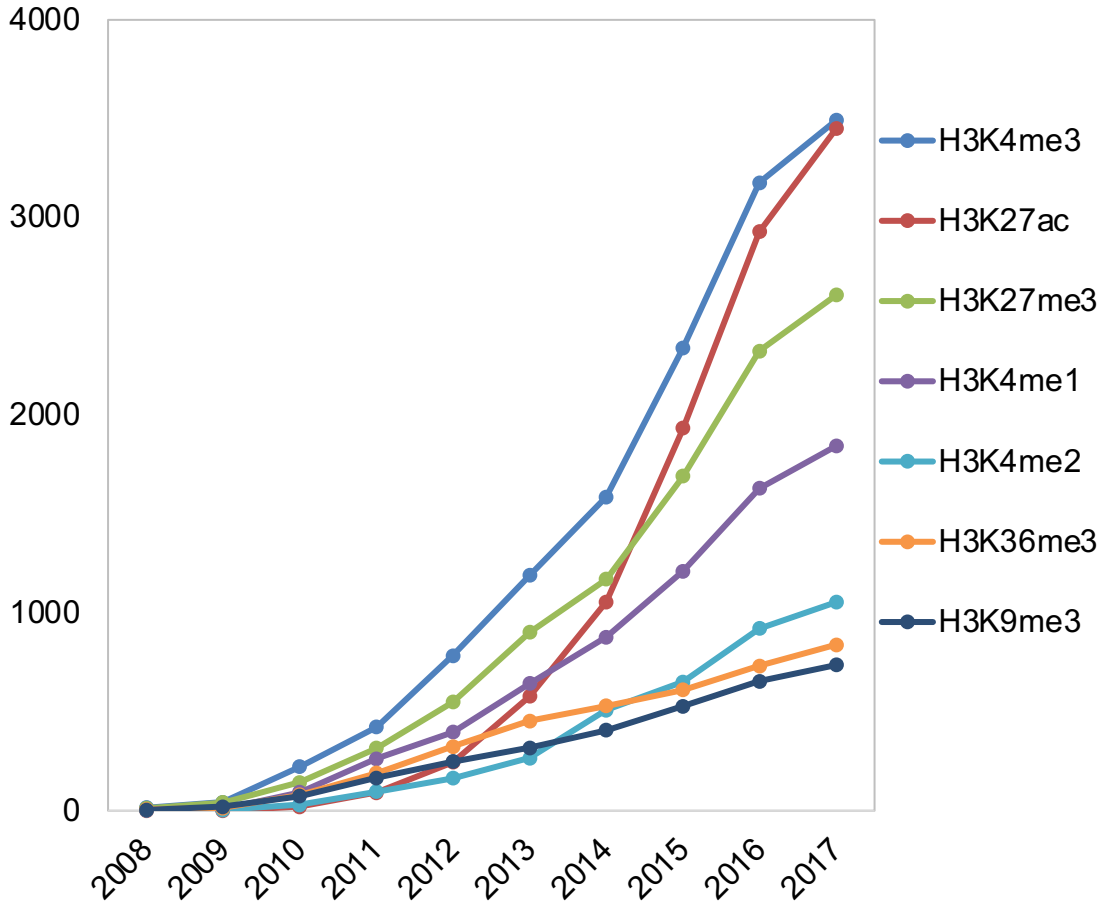
Seq

Schones & Zhao. *Nat. Rev. Genet*. 2008

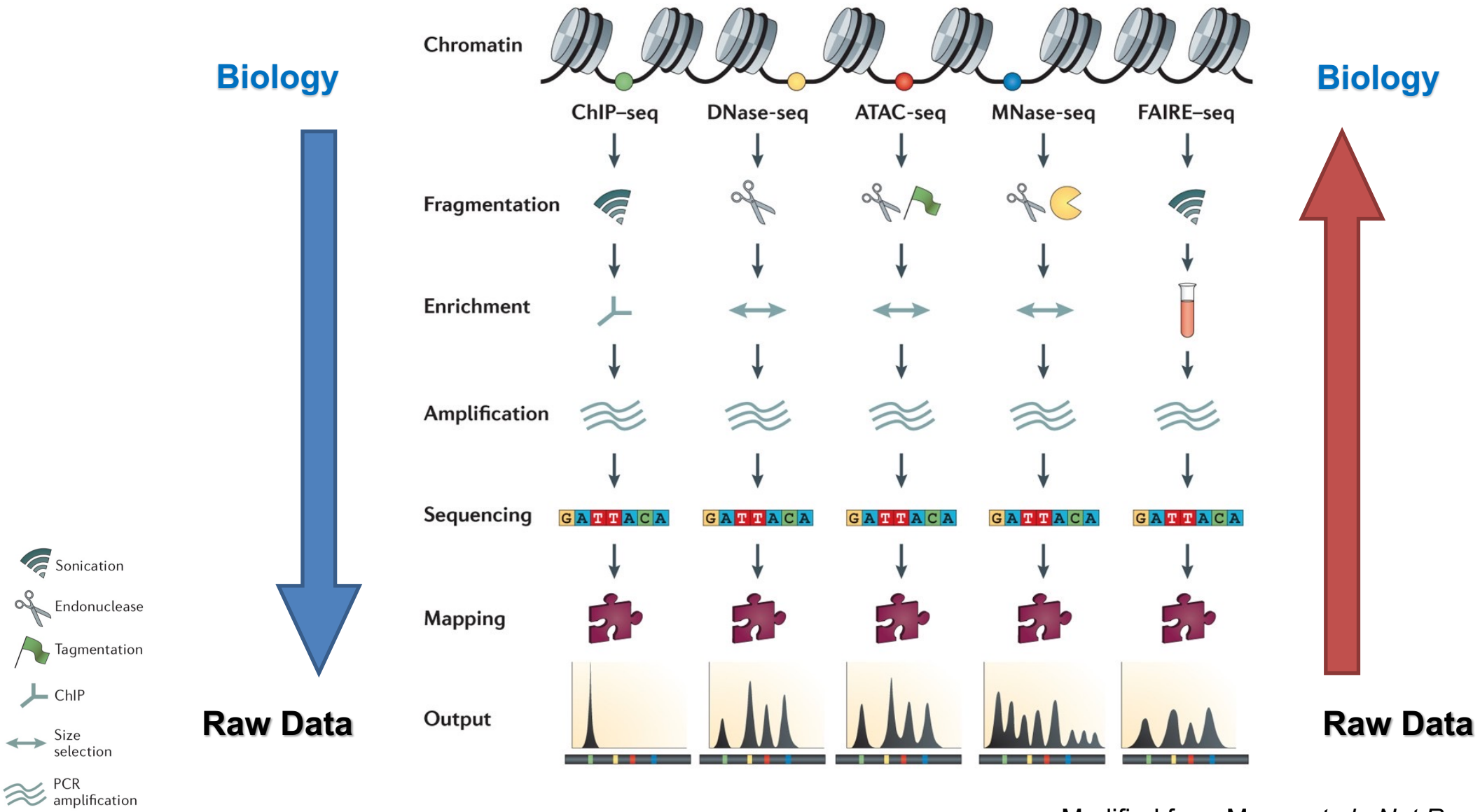
ChIP-seq has become a dominant method for profiling epigenomes



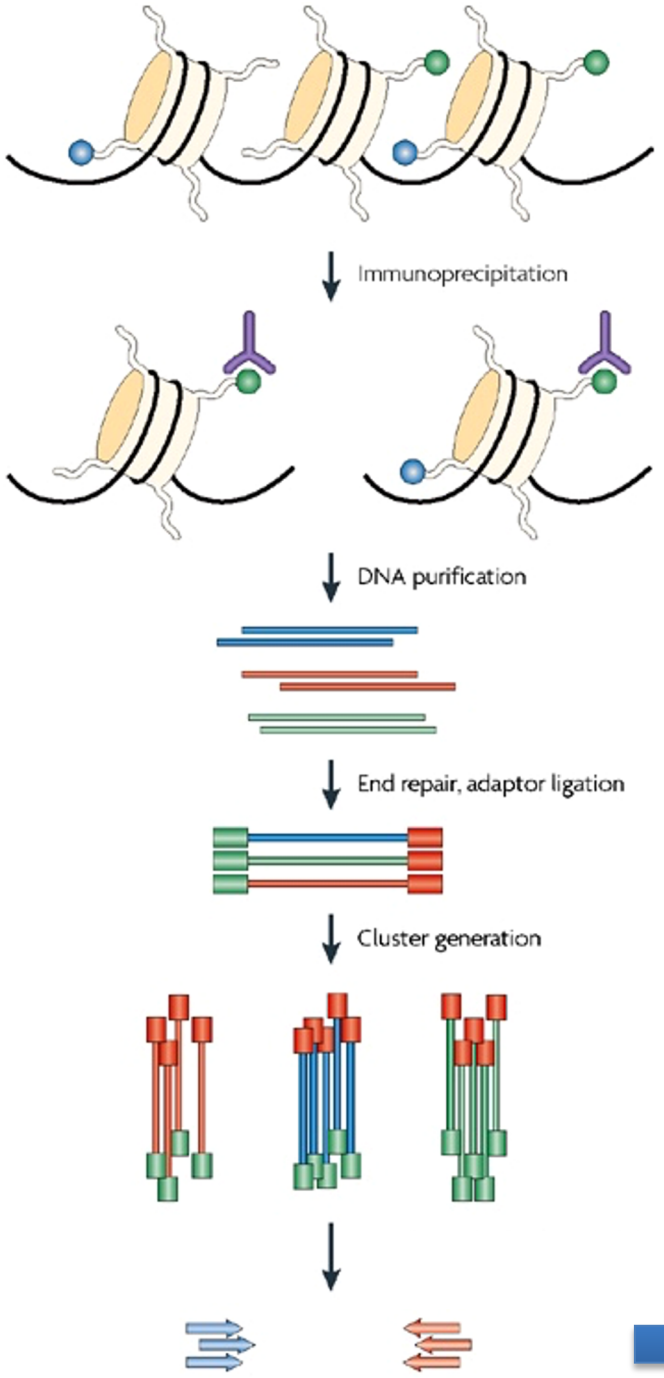
cistrome.org/db



NGS data analysis strategy: Reconstruction of biology



Modified from Mayer *et al.*, *Nat Rev Genet* (2014)



Experimental procedure

Biology

downstream analysis/integration

Peaks (bed)

peak calling

Pile-up for visualization (bedGraph, wig, bigwig)

macs2/ SICER

Non-redundant reads (sam/bam/bed)

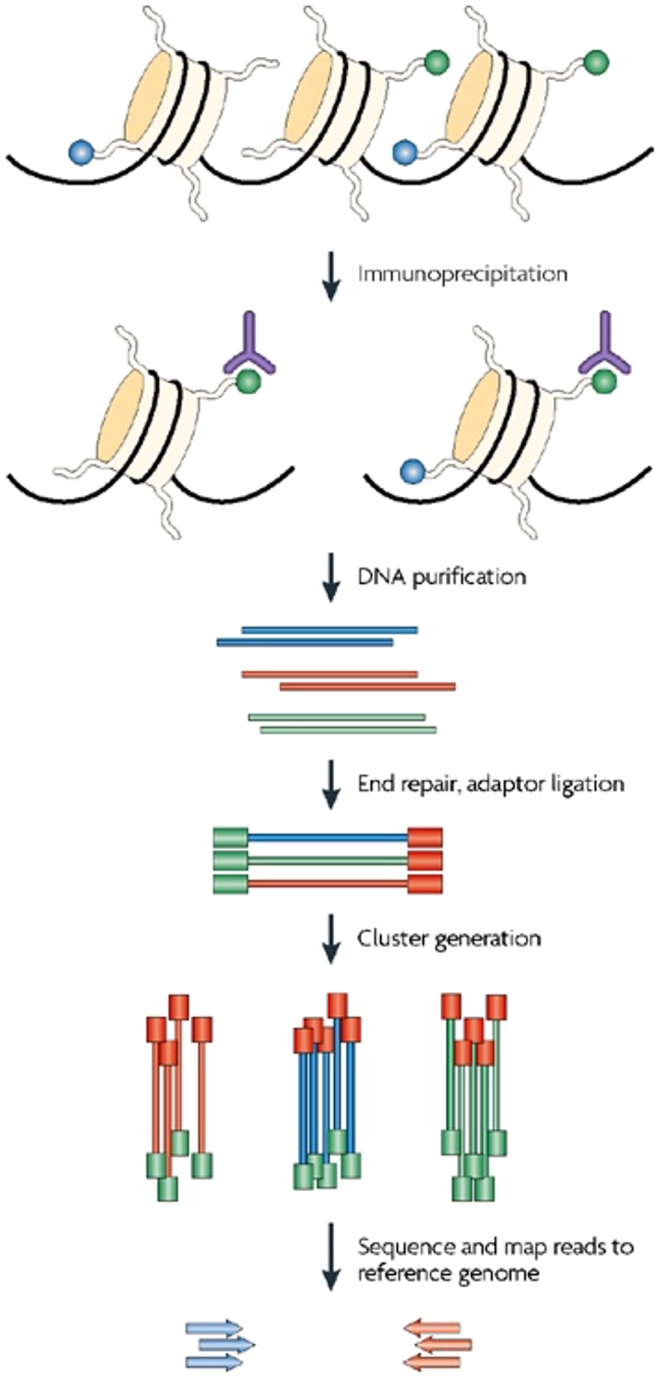
redundancy assessment

Mapped reads (sam/bam/bed)

alignment (**bowtie2**)

Raw sequence reads (fastq)

Computational analysis

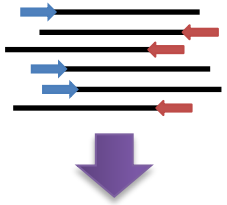


ChIP-seq: Study design

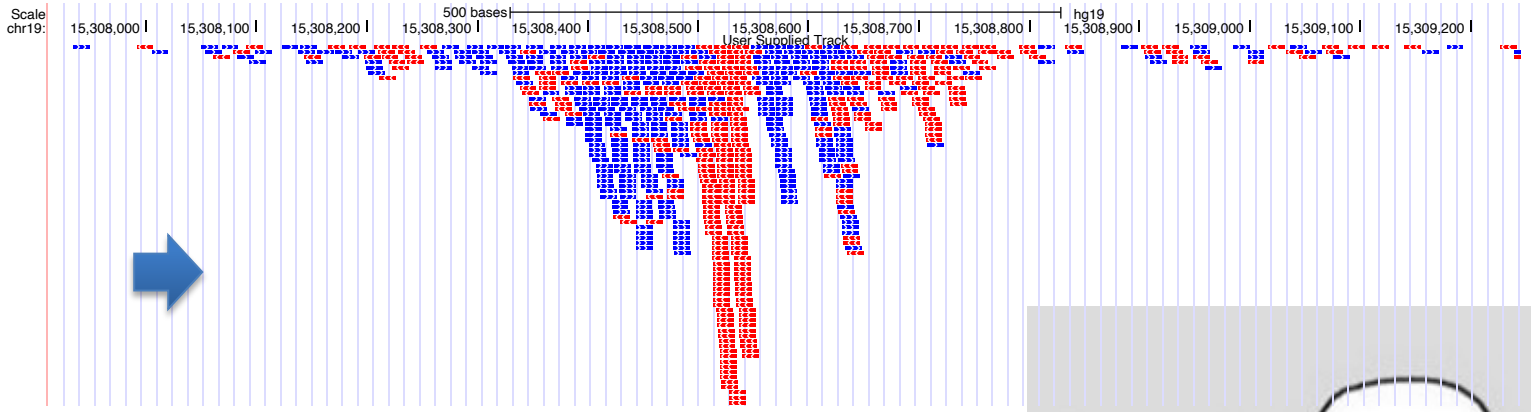
- Background Control: **Input** or **IgG**
  - Input chromatin: sonicated/digested chromatin without immunoprecipitation
  - IgG: “unspecific” immunoprecipitation

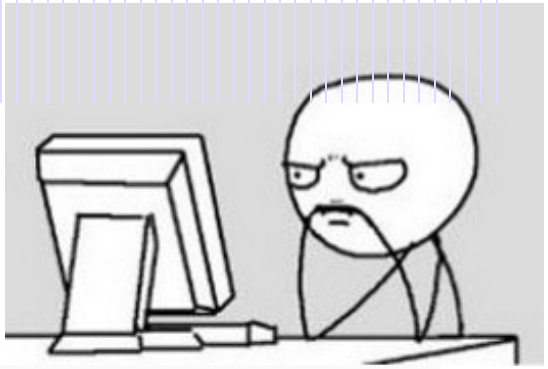
- Study Control:
  - Control exp sample: ChIP + input
  - Treated exp sample: ChIP + input

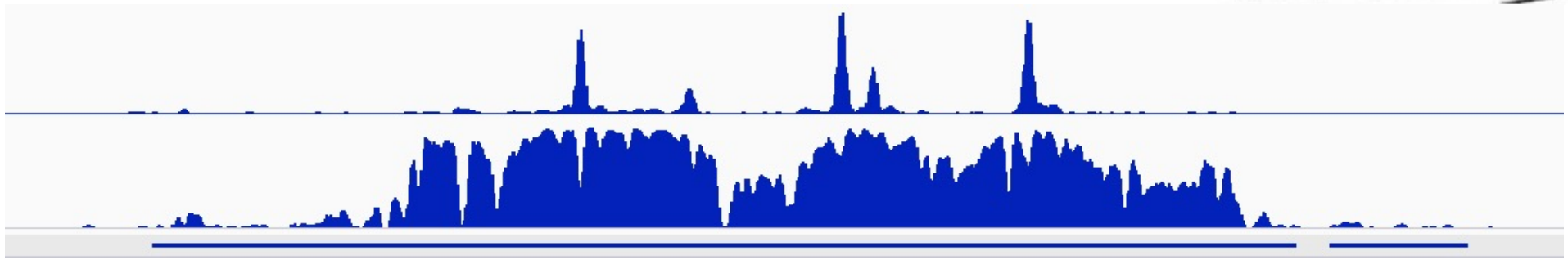
ChIP-seq data analysis overview



```
@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
```

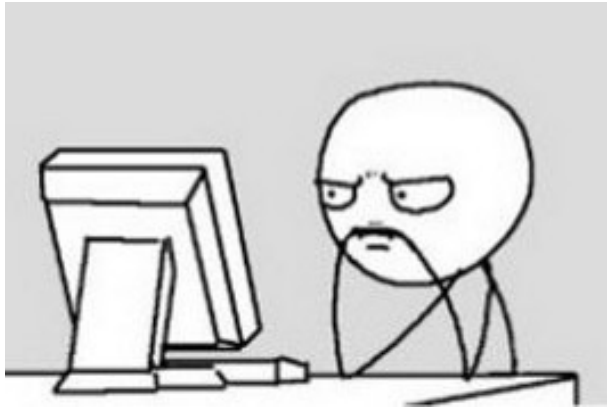






ChIP-seq data analysis overview

- Where in the genome do these sequence reads come from? - Sequence alignment and quality control
- What does the enrichment of sequences mean? - Peak calling
- What can we learn from these data? – Downstream analysis and integration

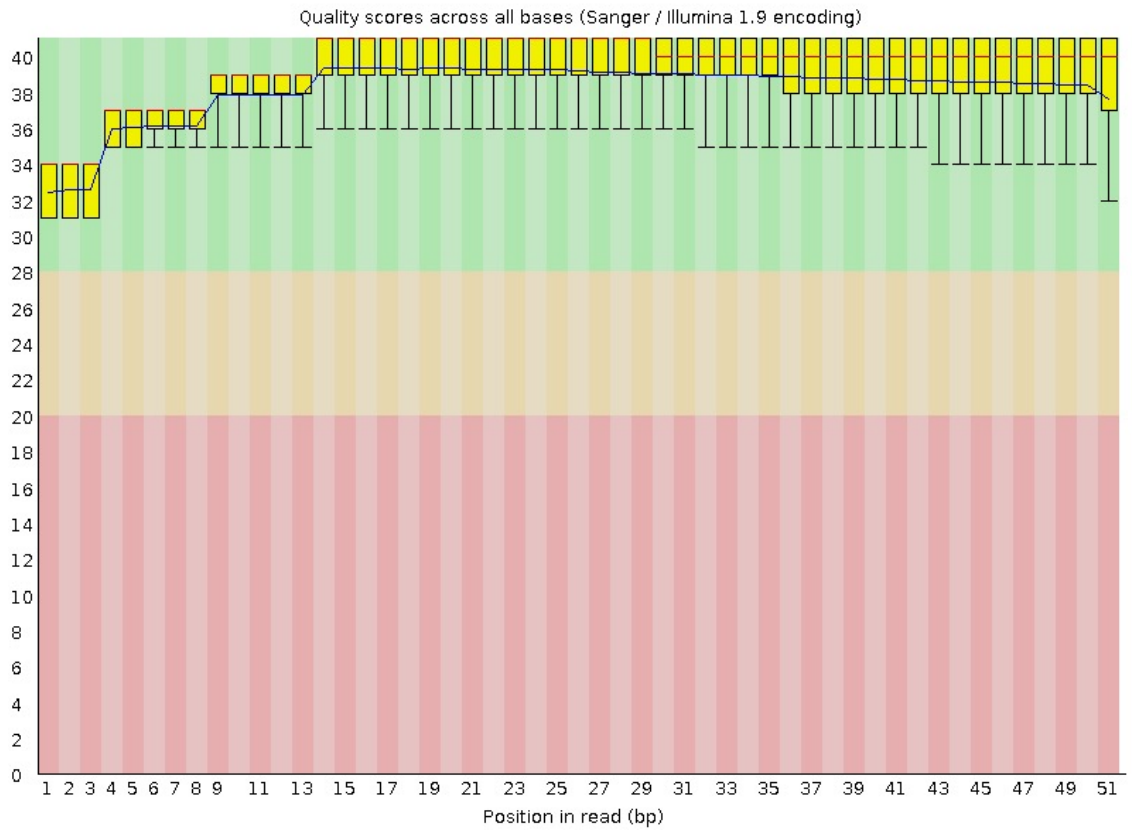


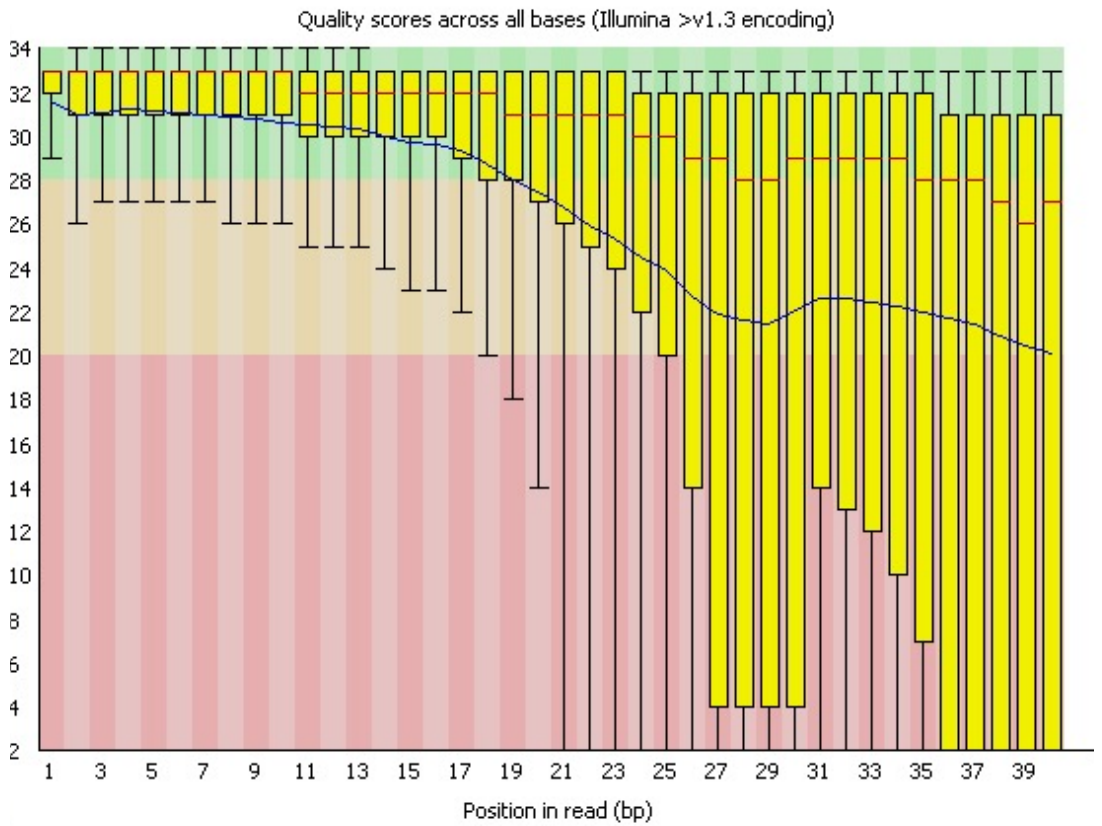
Raw sequence data

- fastq data file:

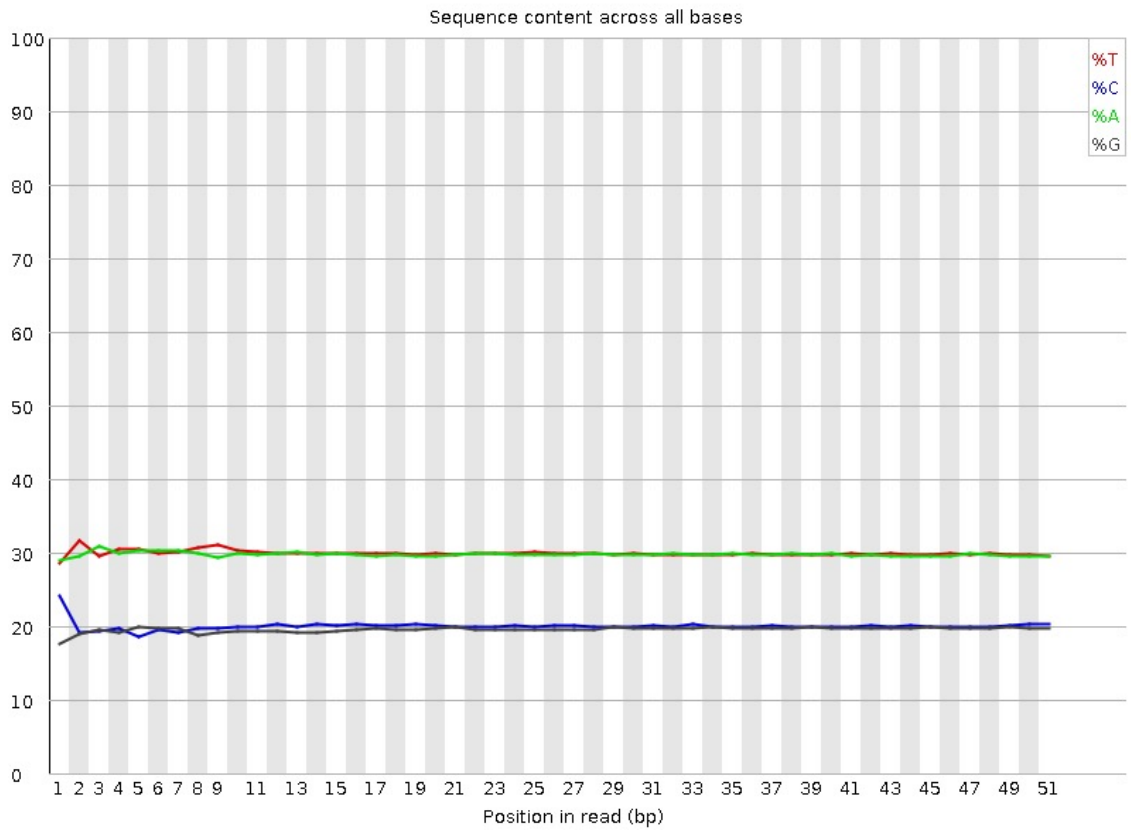
```
@SRR3728822.1 97ZZTR1:422:C57C3ACXX:7:1101:1249:1883/1
NCAAGACCAGTGTCATGAAGCTTTCTCCCTCTTCTAGGAGTTTTACAGCTC
+
#11ADDFFFBFDHGGIJIJJJIEIIJJGHGJFIIHCEHHE?DFHGFHIIJE
@SRR3728822.2 97ZZTR1:422:C57C3ACXX:7:1101:1461:1902/1
NGATTCATAGCTGAATTCTACCAGATGTACAAAGAAGAGCTAGTACCATTA
+
#1=BDFFFHHHHHJJJJIJJJJJJJJJJJJJJIJJJJJGIIJHGIIJIJC
@SRR3728822.3 97ZZTR1:422:C57C3ACXX:7:1101:1479:1943/1
TGAAGATGGCTGAGAACTCCTTAACAGGCAAAATAGGTTTTGTTGGCCGGG
+
C@@FFFFFGHHGGJJJIJJJJJIJJJJIGJFGIJIJJFGHIIIJJIJJIIG
@SRR3728822.4 97ZZTR1:422:C57C3ACXX:7:1101:1287:1958/1
ATATGAACAAACCTTACCTCAGTGGATTCTCAGAACAACCTCTTGAGGTAT
+
CCCFFFFFGHGHHJJIJJJJJJHJJJIJJJIJGIJJJIGHGIJJJGIJEFE
@SRR3728822.5 97ZZTR1:422:C57C3ACXX:7:1101:1515:1796/1
NATTGTGTTTTAGTCTGAAATATCATTTCATGTGGAGAATTCCTTACTGTC
+
#1=DDDFDHHHDHHIIJIIIIJJIHHGHIIGIIIIGIIGIJJIJJIIJJFF
@SRR3728822.6 97ZZTR1:422:C57C3ACXX:7:1101:1585:1807/1
NATAGTTAAAACGGTCTTTCTTTTTGAGATGGAATTTTGCTCTTGTTGCCC
+
#4=DFFFFHHHHHJGIIJJJJJJJJJIIHIJJJIJJJJJJJJJJJJJJJJ
```

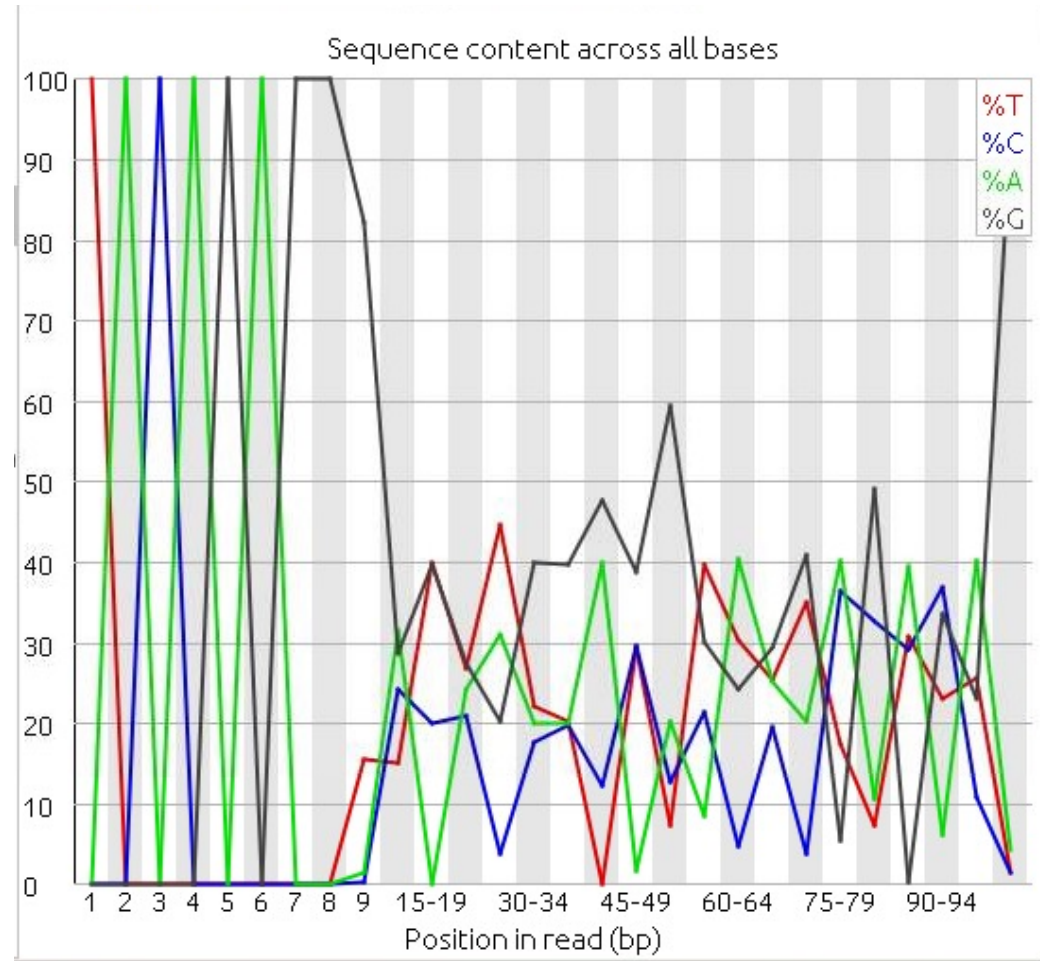
Sequencing quality assessment: fastqc





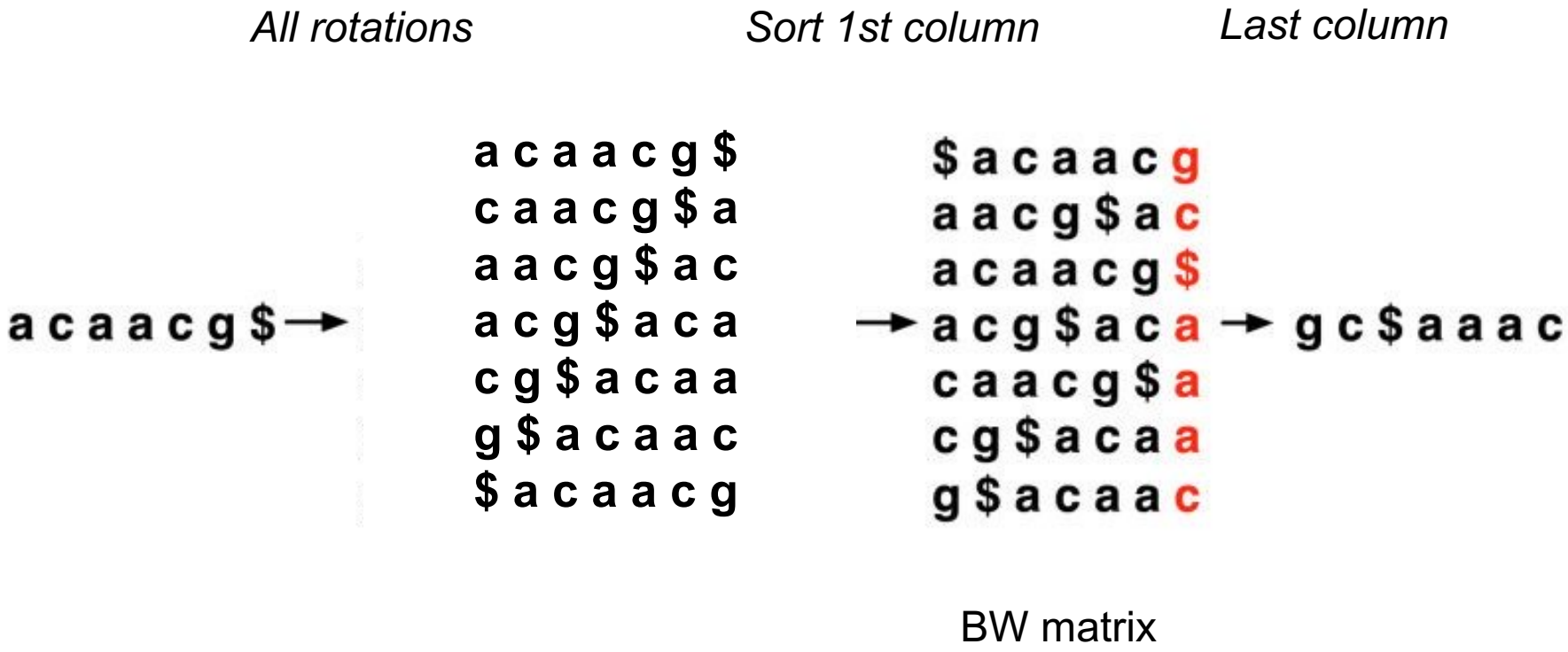
Sequencing quality assessment: fastqc





Burrows-Wheeler transform

Reversible permutation of the characters in a string, originally used for data compression



bowtie, **BW**A

Langmead et al. *Genome Bio* 2009

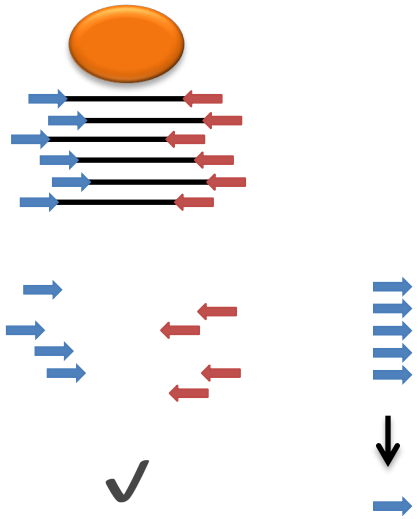
Sequence read mapping: bowtie2/BWA

- alignment of each sequence read: **bowtie2** 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 ✓

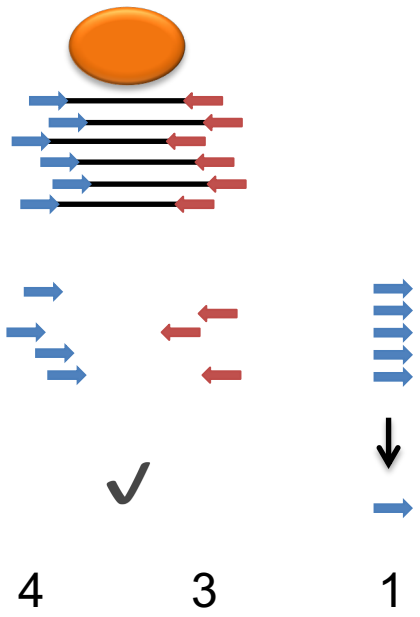
or Quality score

- redundancy assessment:



Langmead et al. 2009,
Zang et al. 2009

Redundancy Control



# mapped reads:	12
# non-redundant reads:	8
# locations w/ reads:	8
# locations w/ 1 read:	7

- Non-redundant rate:

$$\frac{\text{\# non-redundant reads}}{\text{\# mapped reads}}$$

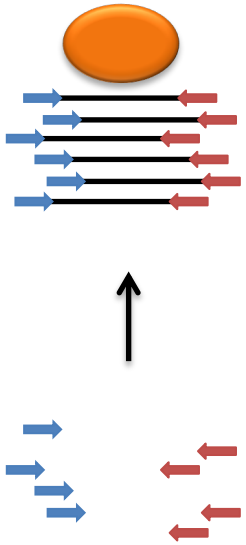
8/12 = 66.7%

- PBC (PCR Bottleneck Coefficient):

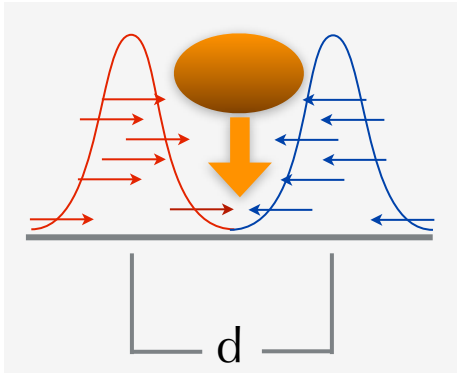
$$\frac{\text{\# locations w/ 1 read}}{\text{\# locations w/ reads}}$$

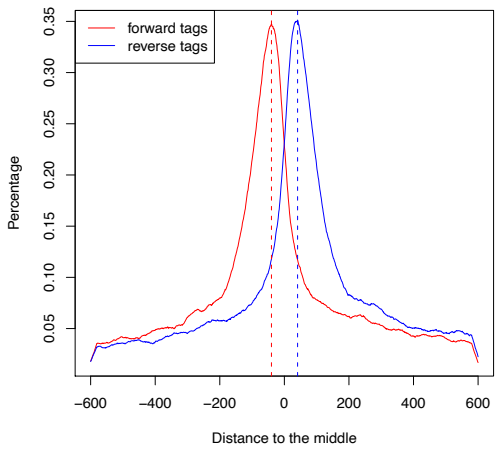
7/8 = 87.5%

DNA fragment size estimation

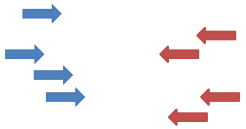


peak model

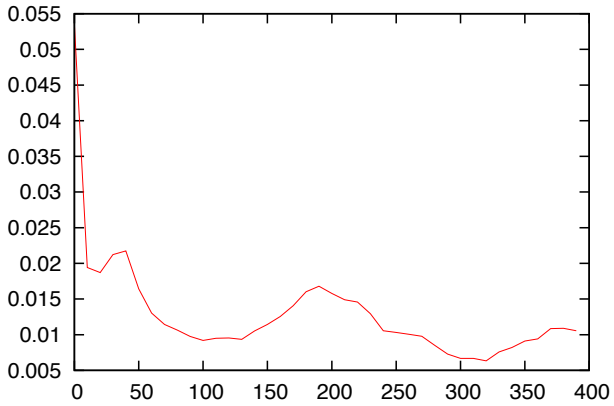




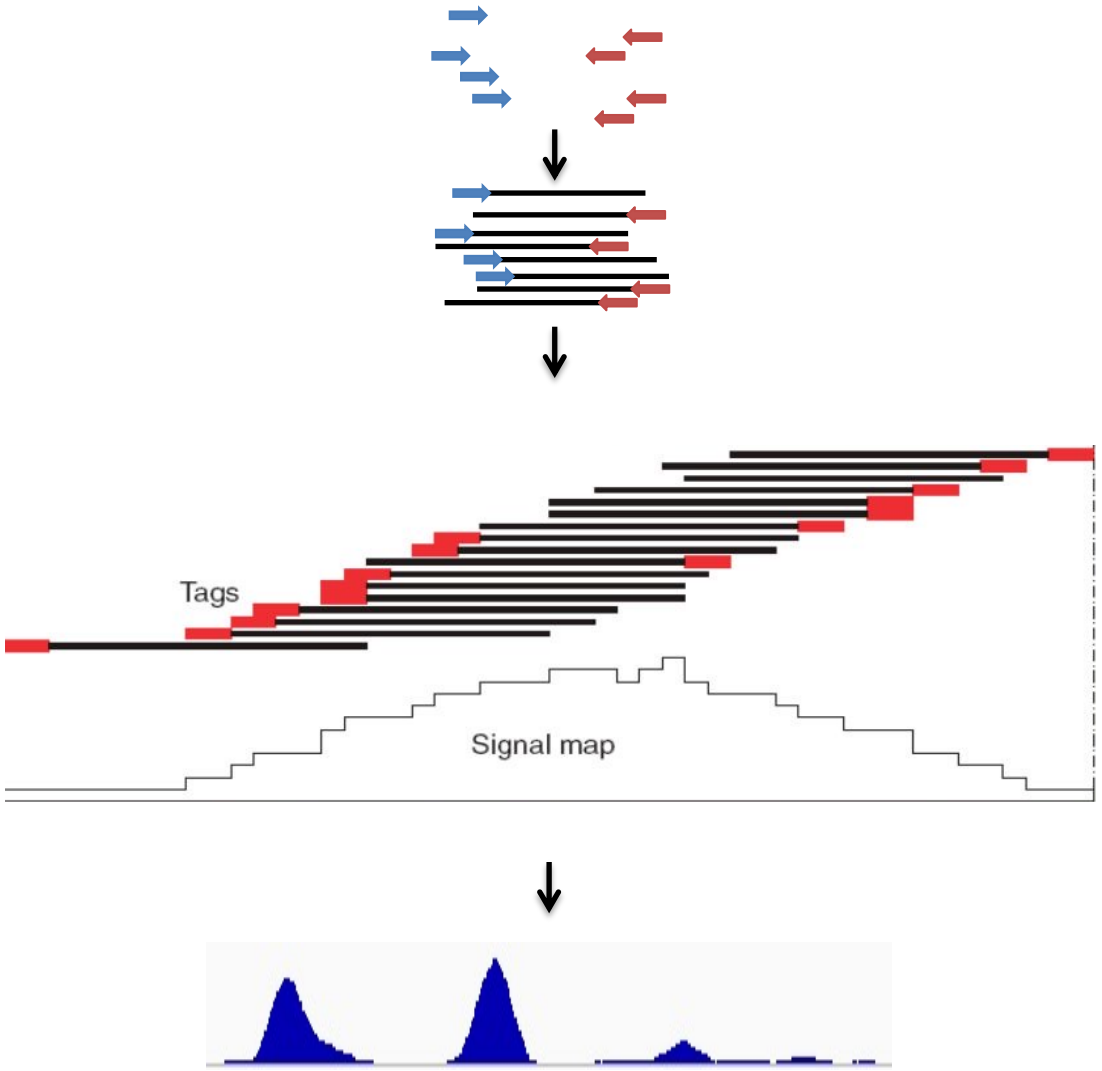
cross-correlation



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



Pile up: visualization



- bedGraph:

```
chr4        10344200        10344250        5
chr4        10344250        10344300        10
chr4        10344300        10344350        25
chr4        10344350        10344400        15
chr4        10344400        10344450        8
```

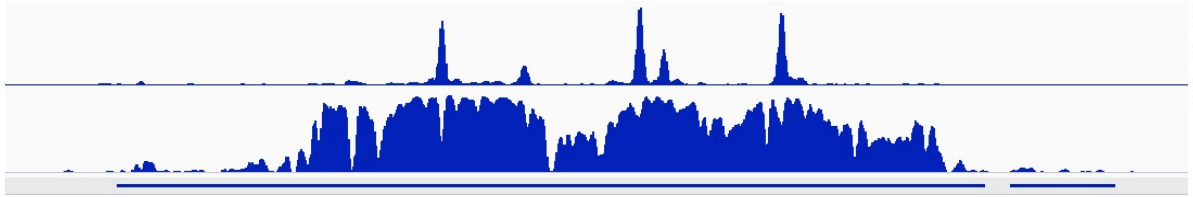
- wiggle:

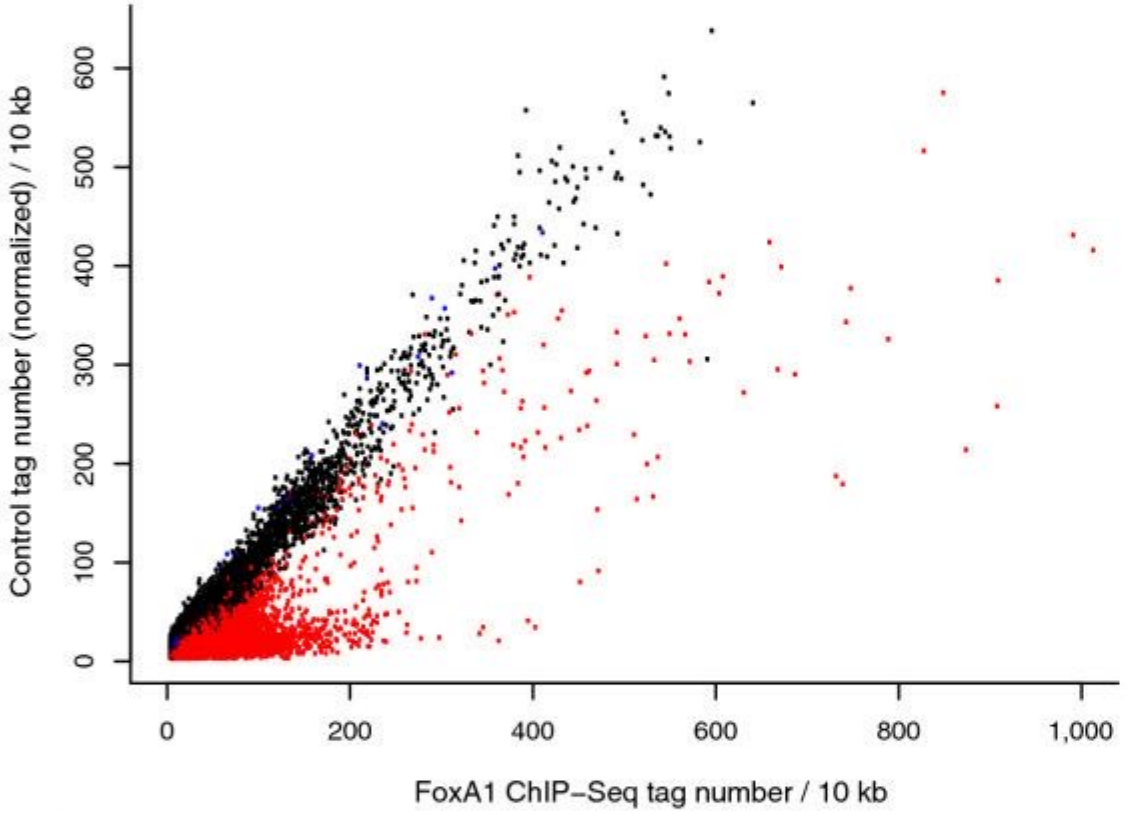
```
track type=wiggle_0
variableStep chrom=chr4 span=50
10344200    5
10344250    10
10344300    25
10344350    15
10344400    8
```

- bigWig: indexed binary format

ChIP-seq: Peak calling

- Goal: Identify regions in the genome enriched for sequence reads:
  - Compared to genomic background
  - Compared to input control



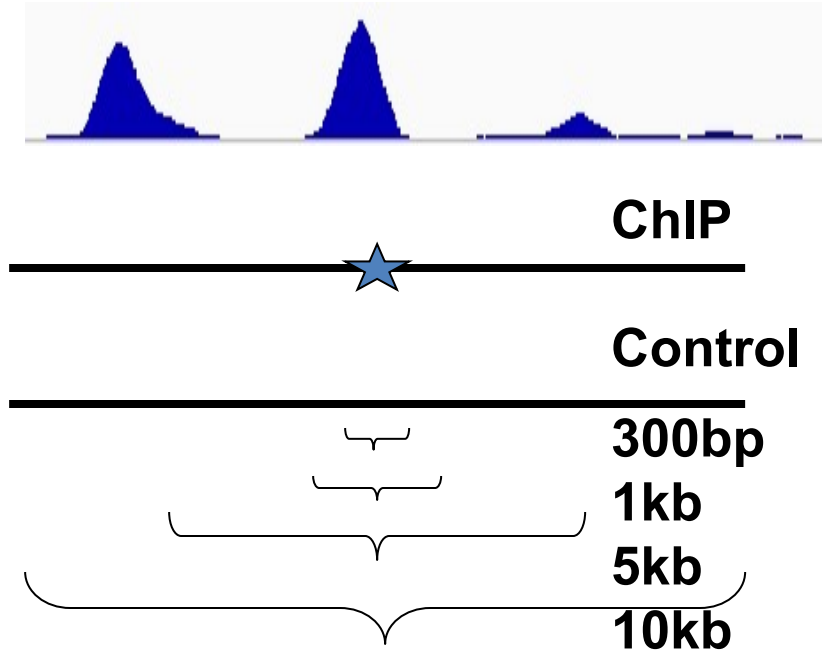


MACS: model

- **M**odel-based **A**nalysis for **C**hIP-**S**eq
- Read distribution along the genome ~ Poisson distribution
  (λ_{BG} = total tag / genome size)
- ChIP-seq show local biases in the genome
  - Chromatin and sequencing bias
  - 200-300bp control windows have to few tags
  - But can look further

Dynamic λ_{local} =
max(λ_{BG}, [λ_{ctrl}, λ_{1k},] λ_{5k}, λ_{10k})

- B-H adjustment to correct for FDR
  - p-value → q-value



Zhang et al, *Genome Bio*, 2008

MACS: Critical input parameters

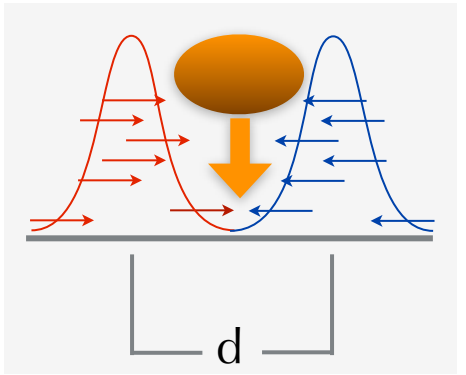
```
macs2 callpeak [-h] -t TFILE [TFILE ...] [-c [CFILE]] [-g GSIZE] [-q QVALUE | -p PVALUE] [--
outdir OUTDIR] [-n NAME] [-B]

-g GSIZE              Effective genome size. It can be 1.0e+9 or 1000000000,
                      or shortcuts:'hs' for human (2.7e9), 'mm' for mouse
                      (1.87e9), 'ce' for C. elegans (9e7) and 'dm' for
                      fruitfly (1.2e8), Default:hs
-q QVALUE
                      Minimum FDR (q-value) cutoff for peak detection.
                      DEFAULT: 0.05. -q, and -p are mutually exclusive.

--outdir OUTDIR       If specified all output files will be written to that
                      directory. Default: the current working directory

-n NAME               Experiment name, which will be used to generate output
                      file names. DEFAULT: "NA"

-B, --bdg             Whether or not to save extended fragment pileup, and
                      local lambda tracks (two files) at every bp into a
                      bedGraph file. DEFAULT: False
```

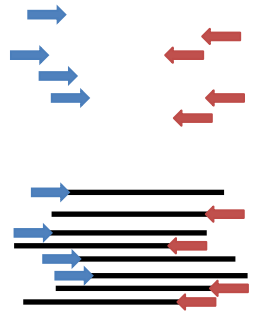
MACS: Output interpretation

```
# This file is generated by MACS version 2.1.2
# Command line: callpeak -t ../bowtie2/AR.sam -g hs -n AR --bdg
# ARGUMENTS LIST:
# name = AR
# format = AUTO
# ChIP-seq file = ['../bowtie2/AR.sam']
# control file = None
# effective genome size = 2.70e+09
# band width = 300
# model fold = [5, 50]
# qvalue cutoff = 5.00e-02
# The maximum gap between significant sites is assigned as the read length/tag size.
# The minimum length of peaks is assigned as the predicted fragment length "d".
# Larger dataset will be scaled towards smaller dataset.
# Range for calculating regional lambda is: 10000 bps
# Broad region calling is off
# Paired-End mode is off
```

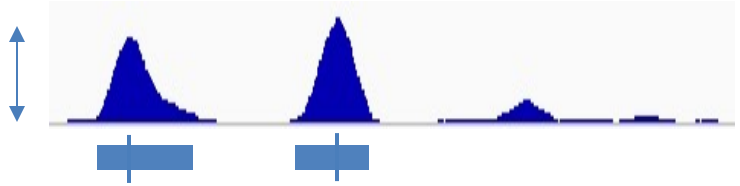
MACS: Output interpretation

```
# tag size is determined as 51 bps
# total tags in treatment: 19442622
# tags after filtering in treatment: 17218335
# maximum duplicate tags at the same position in treatment = 1
# Redundant rate in treatment: 0.11
# d = 141
# alternative fragment length(s) may be 141 bps
```





chr	start	end	length	abs_summit	pileup	-log10(pvalue)	fold_enrichment	-log10(qvalue)	name
chr1	2603	2989	387	2870	18.00	6.68596	3.52825	3.66748	AR_peak_1
chr1	138179	138371	193	138281	18.00	14.90779	7.93021	11.47829	AR_peak_2
chr1	36515	36714	200	36609	16.00	12.59143	7.05394	9.25447	AR_peak_3
chr1	201091	201231	141	201114	10.00	7.58293	5.23859	4.50002	AR_peak_4
chr1	69373	69558	186	69452	18.00	9.61904	4.93737	6.41821	AR_peak_5



MACS: Output interpretation

- Excel

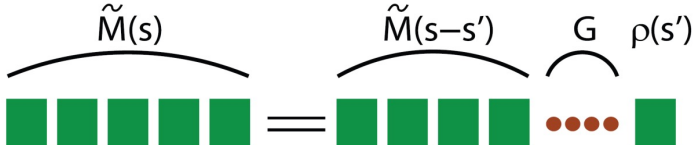
chr	start	end	length	abs_summit	pileup	-log10(pvalue)	fold_enrichment	-log10(qvalue)	name
chr1	2603	2989	387	2870	18.00	6.68596	3.52825	3.66748	AR_peak_1
chr1	138179	138371	193	138281	18.00	14.90779	7.93021	11.47829	AR_peak_2
chr1	36515	36714	200	36609	16.00	12.59143	7.05394	9.25447	AR_peak_3
chr1	201091	201231	141	201114	10.00	7.58293	5.23859	4.50002	AR_peak_4
chr1	69373	69558	186	69452	18.00	9.61904	4.93737	6.41821	AR_peak_5

- narrowPeak

				score		fold	p	q	sm
chr1	591170	591325	AR_peak_290	82	.	6.63900	11.50806	8.21785	25
chr1	629218	629993	AR_peak_291	295	.	3.42374	33.50185	29.54851	636
chr1	630286	630453	AR_peak_292	106	.	2.39458	14.04047	10.64496	81
chr1	630765	631382	AR_peak_293	239	.	3.14283	27.79379	23.97848	480
chr1	631877	632366	AR_peak_294	224	.	3.06645	26.24850	22.47273	380

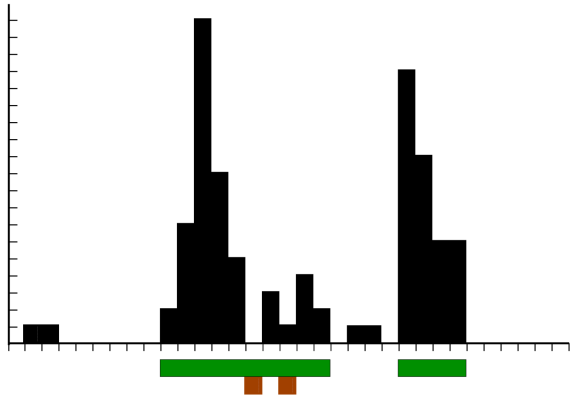
Call broad peaks: SICER

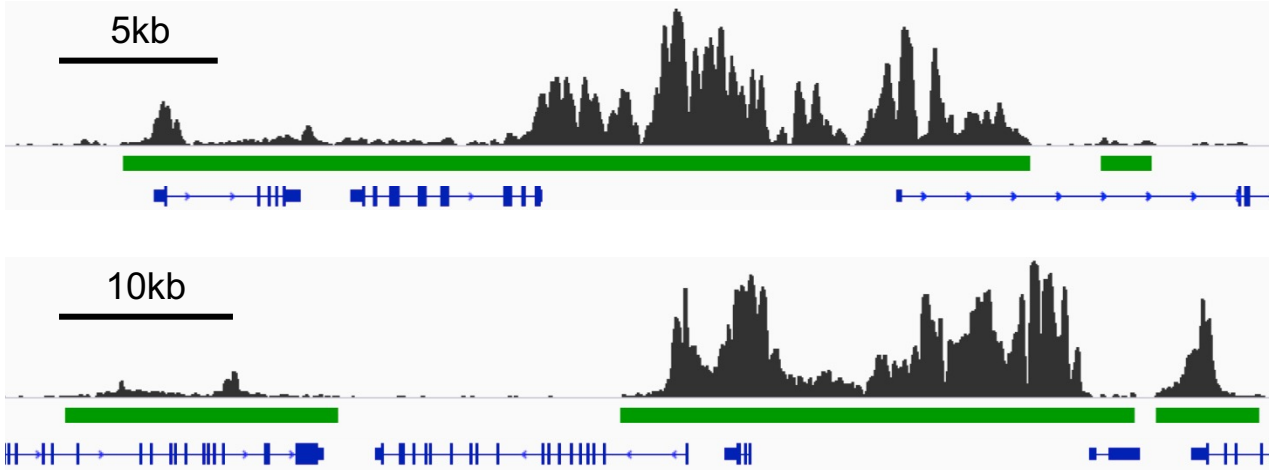
- **S**patial-clustering **I**dentification of **C**hIP-**E**nriched **R**egions



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

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





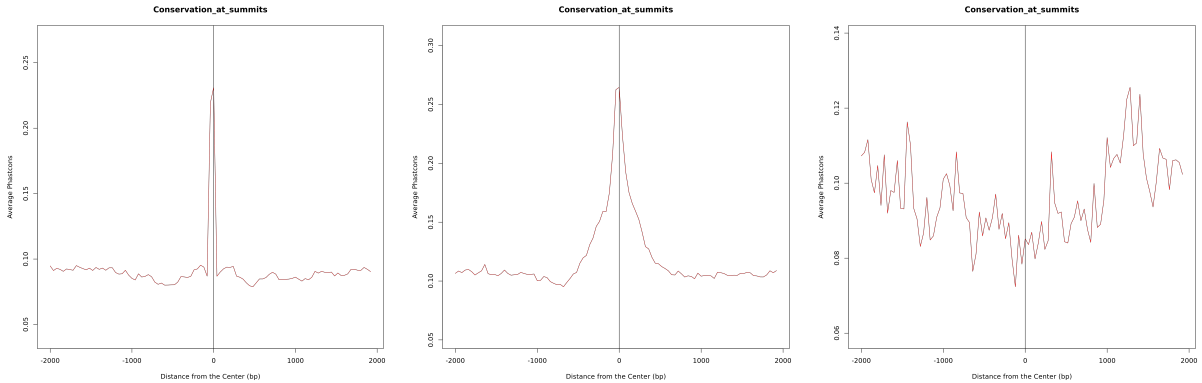
Zang et al. *Bioinformatics* 2009

Quality Control

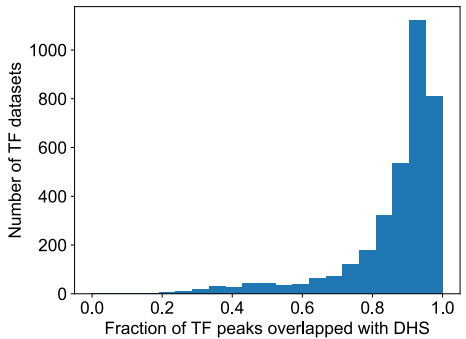
- FRiP (Fraction of Reads in Peaks) score
  - 1-10% for TF is normal

- Number of peaks
  - Number of peaks with high fold-enrichment, e.g, 5, 10, …
  - 2000

- Sequence conservation



- Fraction of peaks within regulatory regions
  - 80%



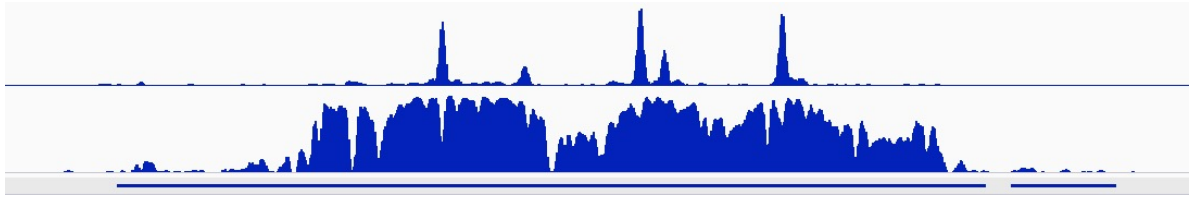
Data formats

- BED:

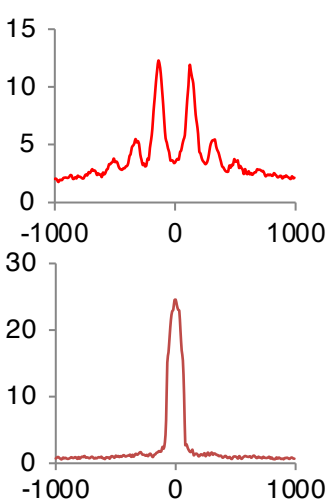
```
chr11    10344210    10344260    255    0    -
chr4     76649430    76649480    255    0    +
chr3     77858754    77858804    255    0    +
chr16    62688333    62688383    255    0    +
chr22    33031123    33031173    255    0    -
```

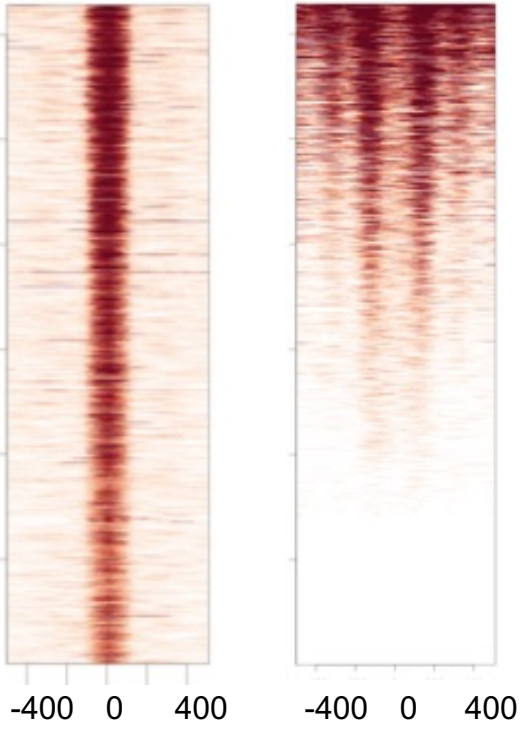
- SAM/BAM: aligned sequencing reads

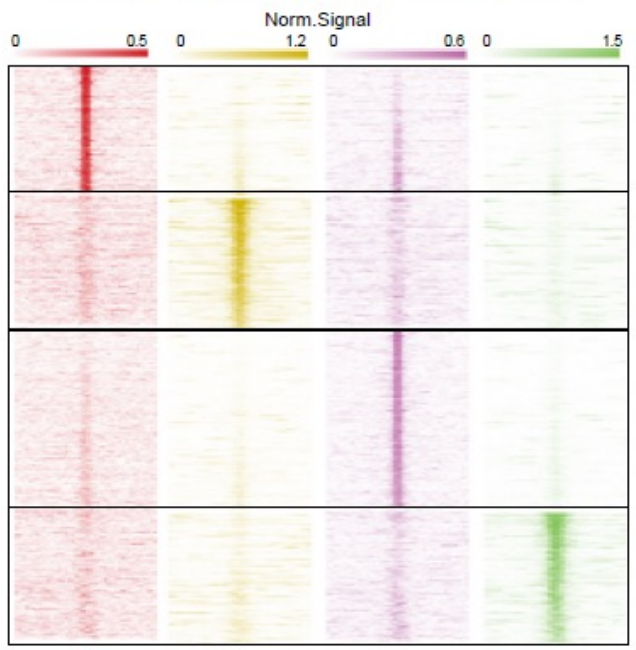
- bedGraph, Wig, bigWig: pile-up profiles for browser visualization

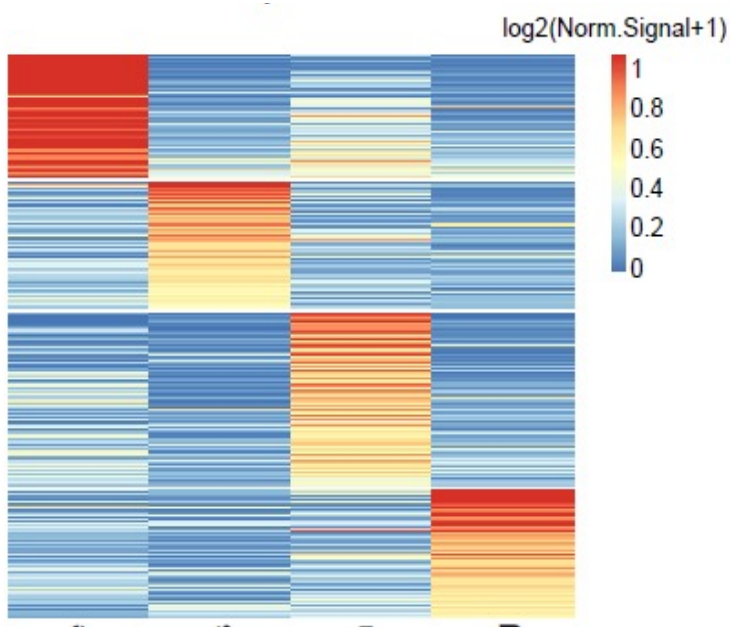


Data visualization

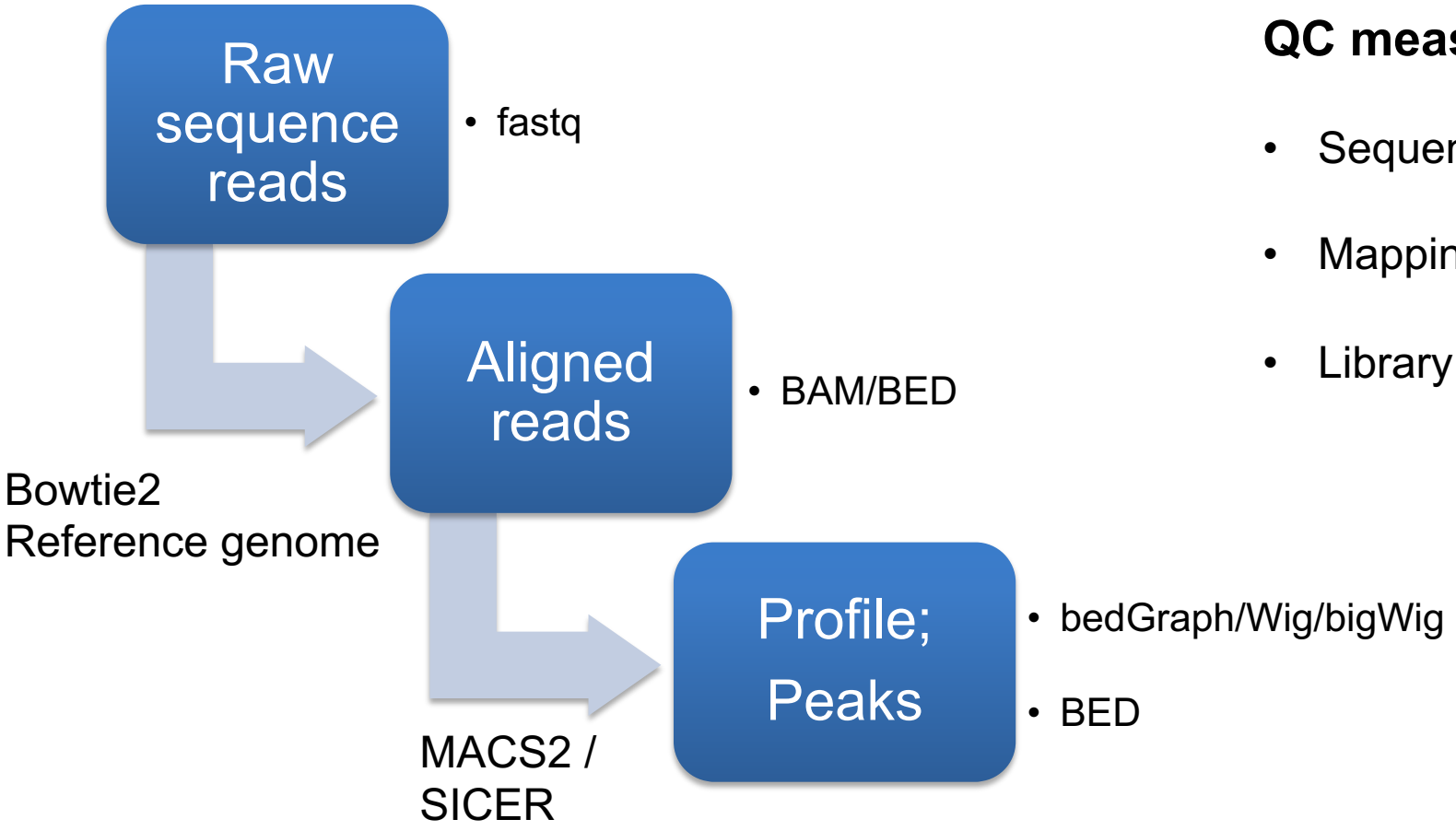






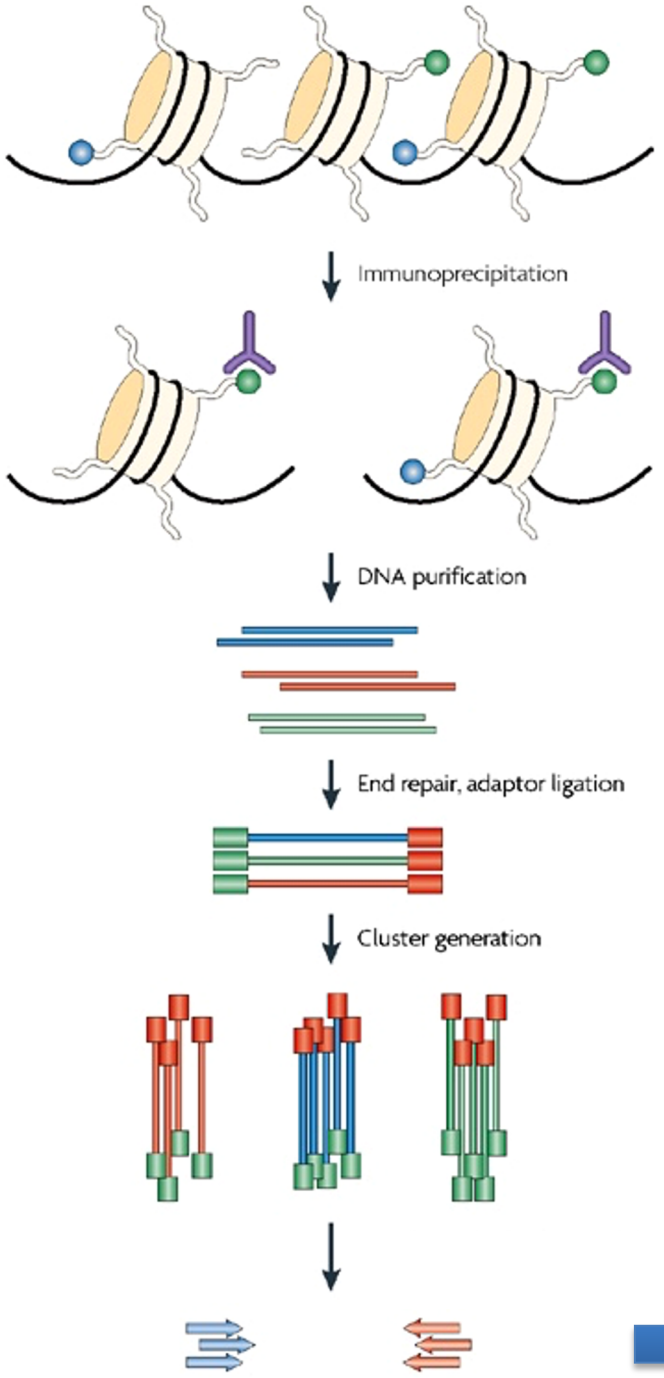


ChIP-seq Data flow and QC summary



QC measures

- Sequence quality (fastqc)
- Mapping quality (uniquely mapped ratio)
- Library complexity (PBC)
- Fold enrichment, peaks
- Signal/Noise (FRIP score)
- Regulatory annotation



Experimental procedure

Biology

downstream analysis/integration

Peaks (bed)

peak calling

Pile-up for visualization (bedGraph, wig, bigwig)

macs2/ SICER

Non-redundant reads (sam/bam/bed)

redundancy assessment

Mapped reads (sam/bam/bed)

alignment (**bowtie2**)

Raw sequence reads (fastq)

Computational analysis

Break

Outline

- 1st Half:
  - NGS introduction
  - NGS data analysis strategy
  - ChIP-seq data analysis

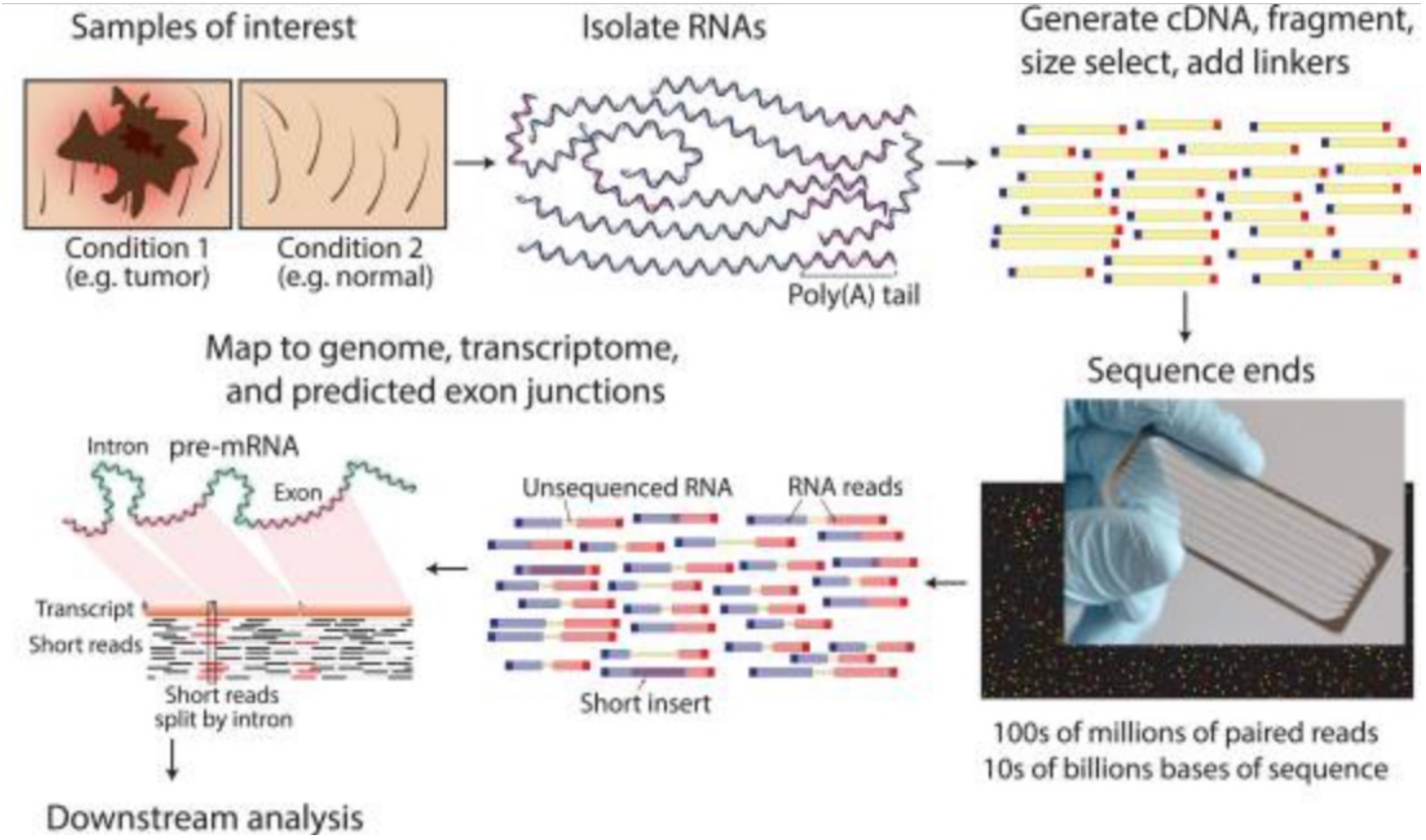
- **2nd Half**:
  - Other NGS data analysis
  - Downstream analysis and data integration
  - Online resources

Other epigenetics NGS data

- RNA-seq
- BS-seq
- Hi-C
- Single-cell techniques (scRNA-seq, scATAC-seq, etc.)

RNA-seq

- Detect gene expression (transcription)
  - Readout of epigenetic changes (especially transcriptional regulation)
- Protocols
  - mRNA (polyA)
  - Total RNA (rRNA depletion)
  - …



Griffith et al., PLoS Comput Biol. 2015

RNA-seq

- QC
  - Exon (gene body) coverage
    - %reads located on exon (genebody) regions
  - Splicing junctions
  - Strand specificity
  - Software
    - RseQC: http://rseqc.sourceforge.net

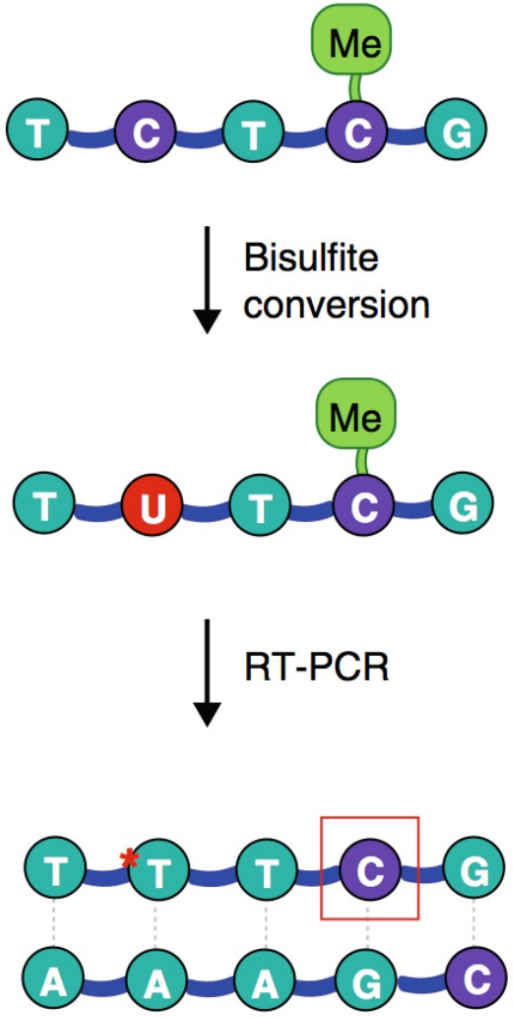
Wang et al., Bioinformatics. 2012

RNA-seq

- Analysis
  - Mapping
    - Hisat2: *Kim et al., Nat Biotechnol. 2019*
  - Gene expression index / reads count
    - Stringtie (novel transcript / splicing events): *Pertea et al., Nat Biotechnol. 2015*
    - HT-seq: *Anders et al., Bioinformatics* 2015
  - Differential expression
    - DESeq2: *Love et al., Genome Biol. 2014*
  - Visualization
    - Pile up the aligned reads (bedtools / ucsc tools)
    - IGV / genome browser view (UCSC genome browser, epigenome browser)

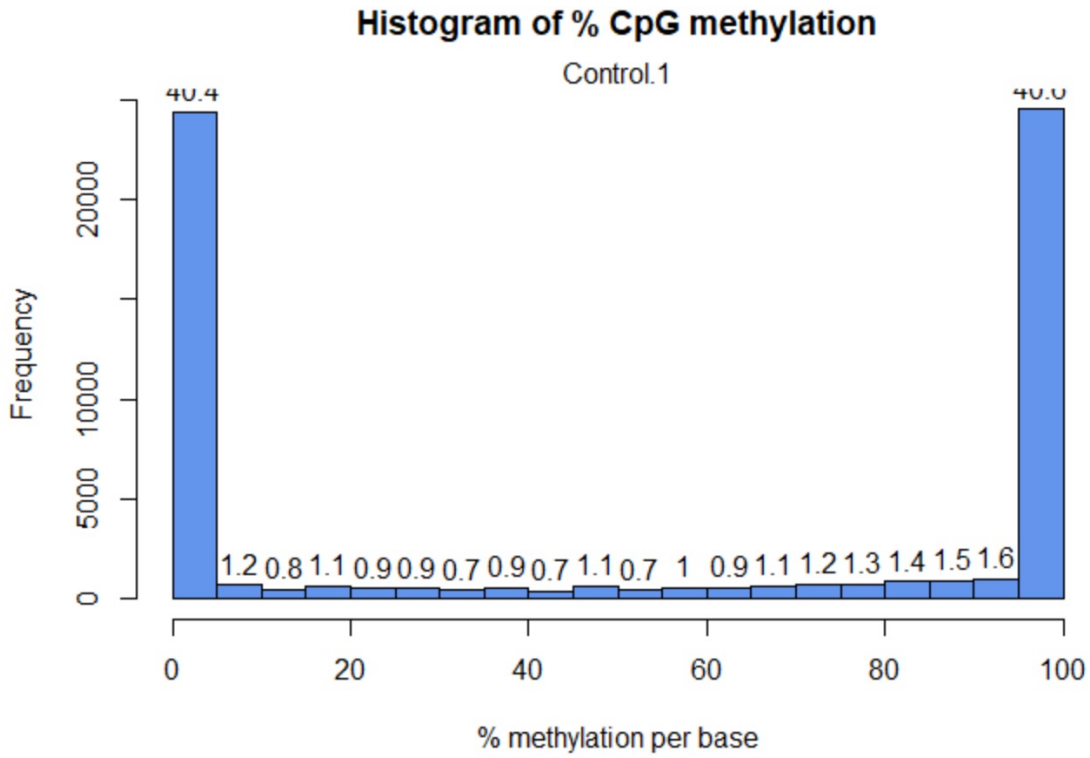
BS-seq

- Detect genome-wide DNA-methylation profiles
- Quantitatively detect DNA-methylation level at CpG sites
- Require higher reads coverage
- Similar to whole genome sequencing (WGS)
- Many variants developed with different advantages
  - RRBS-seq
  - WGBS-seq



BS-seq

- QC
  - Methylation level of lambda DNA
    - Naked DNA with no methylation
    - (whether the BS treatment works)
  - Global CpG methylation status distribution
    - Bi-modal
    - Reads coverage
    - Number of CpG sites with enough reads
    - (whether the detection is reliable)
  - Software:
    - BseQC, MOABS



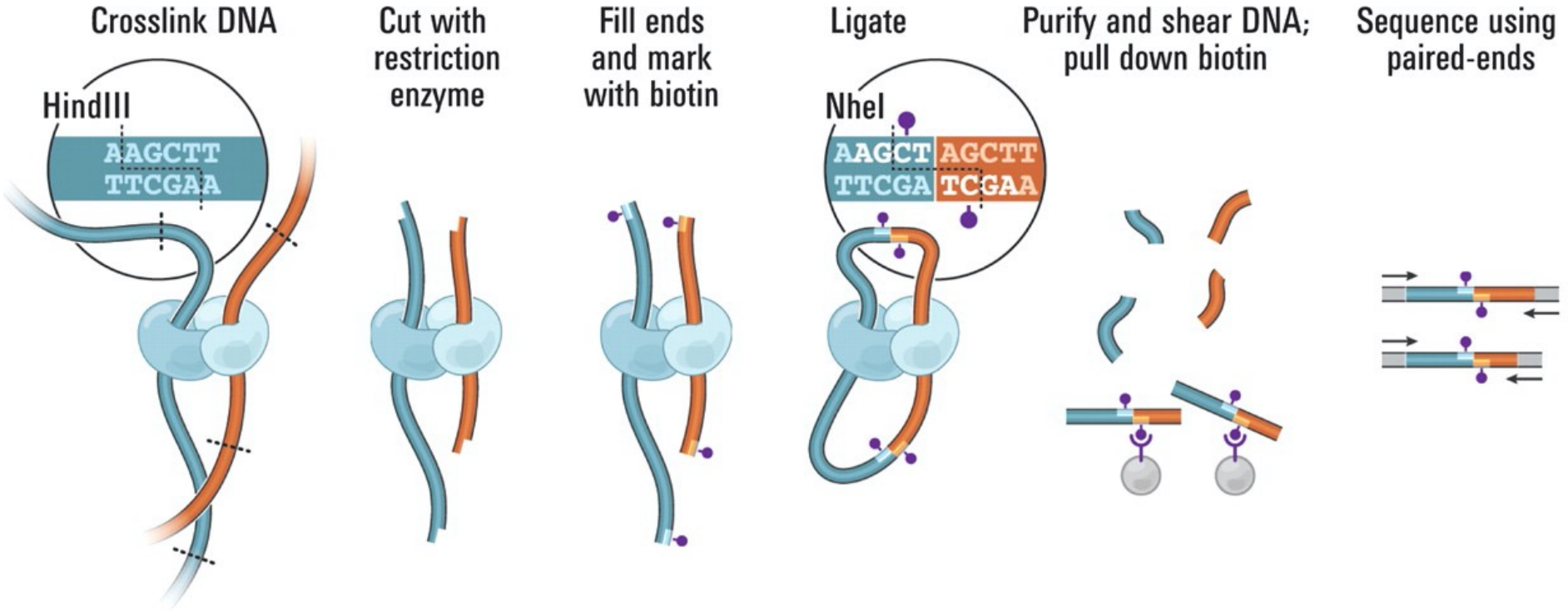
BS-seq

- Analysis
  - Mapping: bsmap
  - Methylation status calling: MOABS
    - *Sun et al., Genome Biol. 2014*
  - Differential methylated region (DMR): MOABS

- Output
  - CpG level methylation table
    - Cutoff on reads coverage (5/10)
  - Region level methylation table
    - Cutoff on qualified CpG (5/10)
  - Bigwig track for genome browser visualization
  - Differential methylated region (DMR)

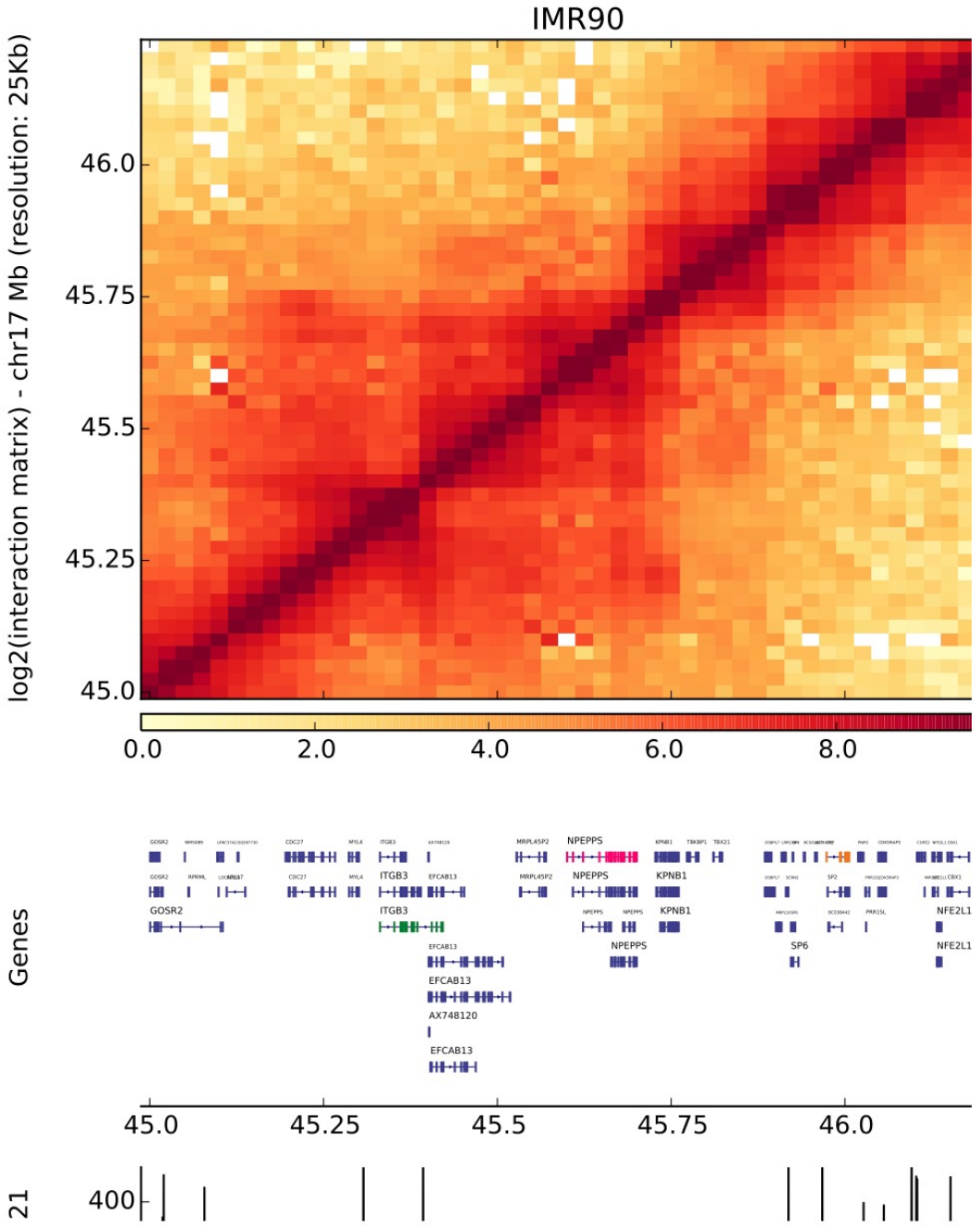
Hi-C

- Chromatin interaction: 3D genome organization
- ChIA-PET
- HiChIP/PLAC-seq



Hi-C

- QC
  - % “Bad” reads pair (invalid ligation product)
    - Self-circle
    - Dangling-end
    - Inter-chromosomal contact
      (not necessarily bad)
  - % short range / long ranges interaction
- Analysis
  - Interaction matrix
  - Heatmap visualization
- Software:
  - HiC-pro (QC + process)
  - Juicer (visualization)



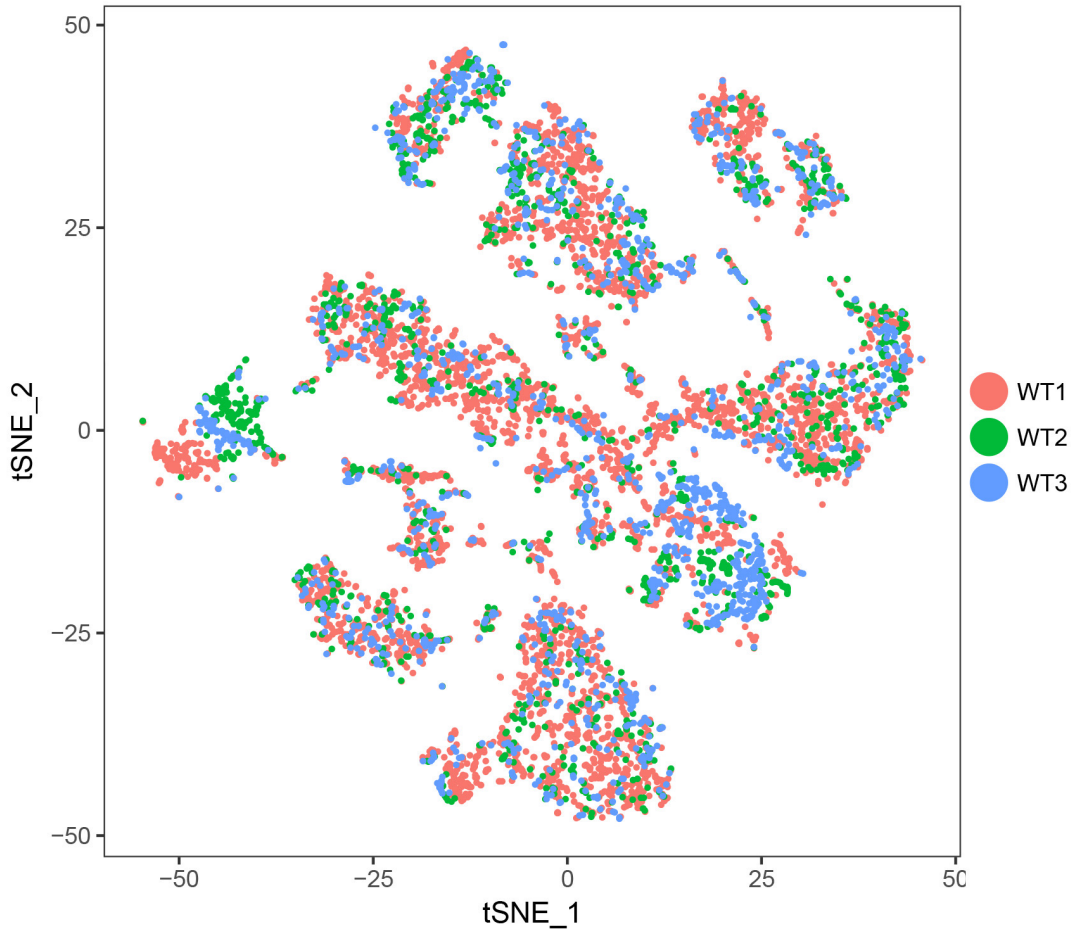
Akdemir et al., Genome Biol. 2015

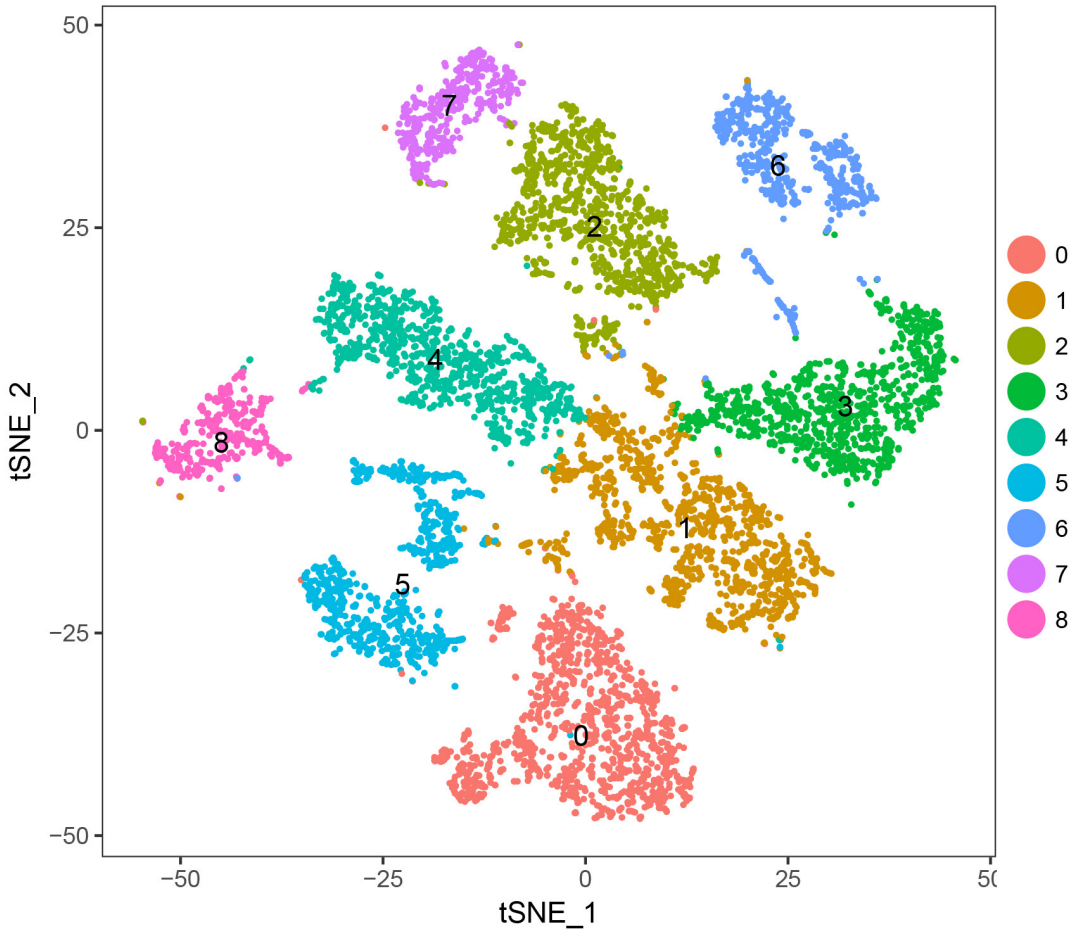
Single-cell data analysis (scRNA-seq or scATAC-seq)

- QC:
  - Sequencing quality, sequencing depth, unique UMI, cells with enough read count etc.

- Analysis:
  - Cell – feature count matrix
  - Dimensionality reduction
  - Clustering vs. t-SNE/UMAP visualization
  - Downstream analysis

Clustering vs. Visualization





Downstream analysis and integration

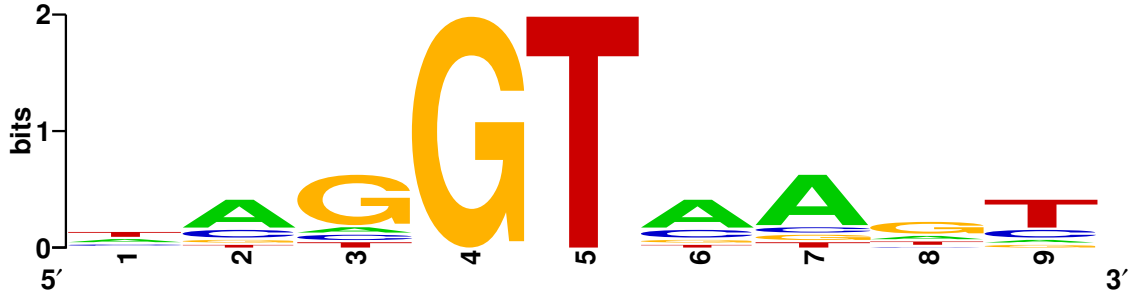
1. DNA sequences at the peaks: motif discovery
2. Annotation of the peaks
3. Integration with other omics data/information for functional analyses

Position weight matrix (PWM) representation of DNA sequence motifs

GAGGTAAAC
TCCGTAAGT
CAGGTTGGA
ACAGTCAGT
TAGGTCATT
TAGGTACTG
ATGGTAACT
CAGGTATAC
TGTGTGAGT
AAGGTAAGT

$$M = \begin{matrix} A \\ C \\ G \\ T \end{matrix} \begin{bmatrix} 3 & 6 & 1 & 0 & 0 & 6 & 7 & 2 & 1 \\ 2 & 2 & 1 & 0 & 0 & 2 & 1 & 1 & 2 \\ 1 & 1 & 7 & 10 & 0 & 1 & 1 & 5 & 1 \\ 4 & 1 & 1 & 0 & 10 & 1 & 1 & 2 & 6 \end{bmatrix}$$

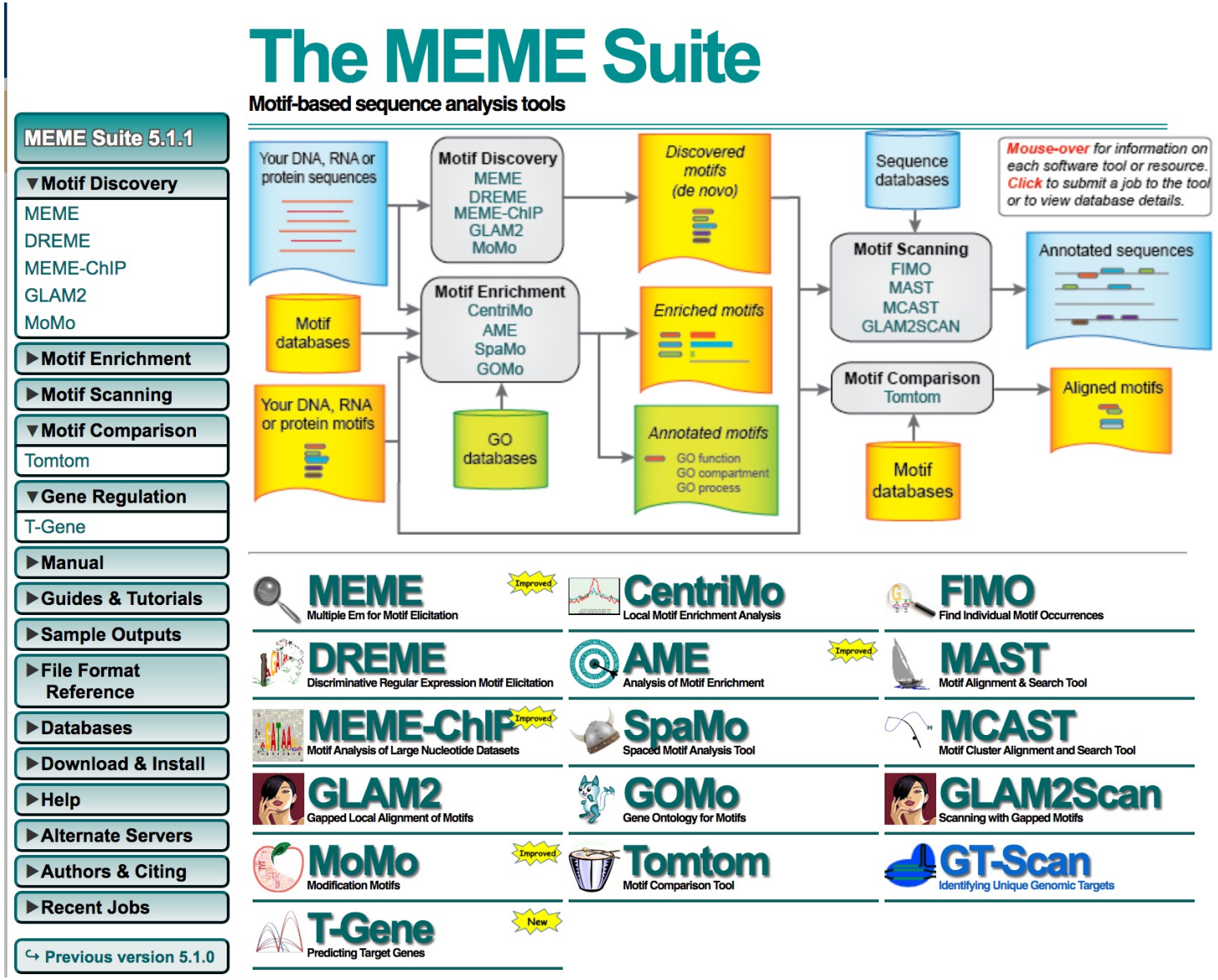
$$M = \begin{matrix} A \\ C \\ G \\ T \end{matrix} \begin{bmatrix} 0.3 & 0.6 & 0.1 & 0.0 & 0.0 & 0.6 & 0.7 & 0.2 & 0.1 \\ 0.2 & 0.2 & 0.1 & 0.0 & 0.0 & 0.2 & 0.1 & 0.1 & 0.2 \\ 0.1 & 0.1 & 0.7 & 1.0 & 0.0 & 0.1 & 0.1 & 0.5 & 0.1 \\ 0.4 & 0.1 & 0.1 & 0.0 & 1.0 & 0.1 & 0.1 & 0.2 & 0.6 \end{bmatrix}$$



$$R_i = \log_2(4) - H_i$$

$$H_i = -\sum_b f_{b,i} \times \log_2 f_{b,i}$$

MEME (meme-suite.org)



HOMER (homer.ucsd.edu)

Not Secure | homer.ucsd.edu/homer/introduction/basics.html

HOMER

Software for motif discovery and ChIP-Seq analysis

Introduction to HOMER

The best way to learn about HOMER is to go through the tutorial pages. We've tried to spell out what happens in each step and explain the "why". A brief description of the Motif Finding component of HOMER is found below. Explanation of the sequencing analysis components of HOMER are integrated into the tutorials.

General Introduction to Motif Discovery with HOMER

HOMER is a collection of tools that are commonly needed for the analysis of gene expression profiling (microarray) and genome-wide location analysis experiments (ChIP-Seq or ChIP-Chip). There are also routines for other types of sequencing experiments, such as DNase-Seq or GRO-Seq.

Some of the things HOMER does NOT DO is find differentially expressed genes (although it has some routines to help with this), cluster gene expression profiles, or search for all the instances Transfac motifs in order to make you hopelessly confused!!! The idea was not to completely reinvent the wheel if possible.

Unfortunately, HOMER must be run as a command-line tool, and may be difficult to use if you are new to UNIX. While commands have been distilled to be as simple and user-friendly as possible, basic knowledge of the UNIX environment and file system is critical (but can probably be learned quickly after typing "unix tutorial" into google). I am proud to say that may of the people using HOMER are completely new to UNIX, so it is indeed possible. In addition, a spreadsheet program (i.e. EXCEL) is needed to graph and visualize some of the results produced by HOMER.

Below is a description of how motif analysis is executed with HOMER. Documentation describing the steps of analysis for Next-Gen Sequencing (or genomic position analysis) or Microarrays (gene-based analysis) are covered in separate sections.

***De Novo* Motif Discovery Strategy**

GREAT (great.stanford.edu)

GREAT predicts functions of *cis*-regulatory regions.

1. **Input:** A set of Genomic Regions (such as transcription factor binding events identified by ChIP-Seq).

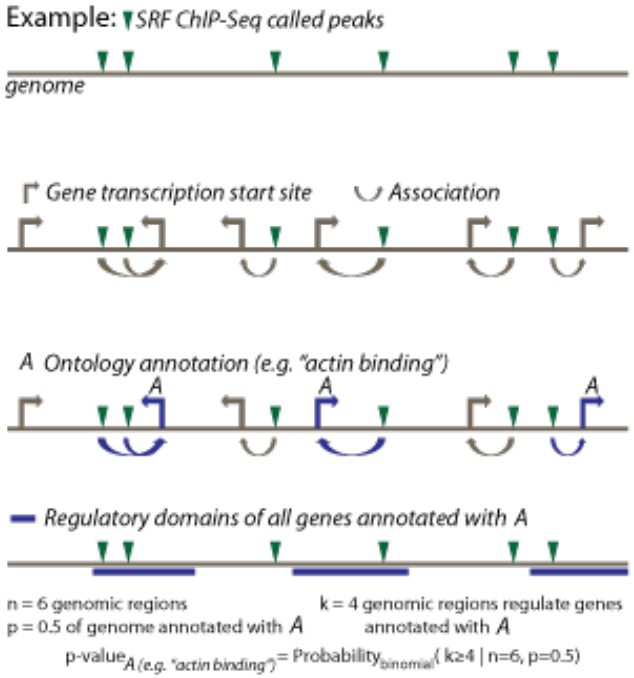
2. GREAT associates both proximal and distal input Genomic Regions with their putative target genes.

3. GREAT uses gene Annotations from numerous ontologies to associate genomic regions with annotations.

4. GREAT calculates statistical Enrichments for associations between Genomic Regions and Annotations.

5. **Output:** Annotation terms that are significantly associated with the set of input Genomic Regions.

6. Users can create UCSC custom tracks from term-enriched subsets of Genomic Regions. Any track can be directly submitted to GREAT from the UCSC Table Browser.



ChIPseeker: an R/Bioconductor package

Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

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Home » Bioconductor 3.10 » Software Packages » ChIPseeker

ChIPseeker

platforms all | rank 123 / 1823 | posts 2 / 0 / 1 / 0 | in Bioc 6 years
build warnings | updated since release | dependencies 152

DOI: 10.18129/B9.bioc.ChIPseeker

ChIPseeker for ChIP peak Annotation, Comparison, and Visualization

Bioconductor version: Release (3.10)

This package implements functions to retrieve the nearest genes around the peak, annotate genomic region of the peak, statstical methods for estimate the significance of overlap among ChIP peak data sets, and incorporate GEO database for user to compare the own dataset with those deposited in database. The comparison can be used to infer cooperative regulation and thus can be used to generate hypotheses. Several visualization functions are implemented to summarize the coverage of the peak experiment, average profile and heatmap of peaks binding to TSS regions, genomic annotation, distance to TSS, and overlap of peaks or genes.

Author: Guangchuang Yu [aut, cre], Yun Yan [ctb], Hervé Pagès [ctb], Michael Kluge [ctb], Thomas Schwarzl [ctb], Zhougeng Xu [ctb]

Maintainer: Guangchuang Yu <guangchuangyu at gmail.com>

Citation (from within R, enter `citation("ChIPseeker")`):

Yu G, Wang L, He Q (2015). "ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization." *Bioinformatics*, **31**(14), 2382-2383. doi: 10.1093/bioinformatics/btv145.

Documentation »

Bioconductor

- Package vignettes and manuals.
- Workflows for learning and use.
- Course and conference material.
- Videos.
- Community resources and tutorials.

R / CRAN packages and documentation

Support »

Please read the posting guide. Post questions about Bioconductor to one of the following locations:

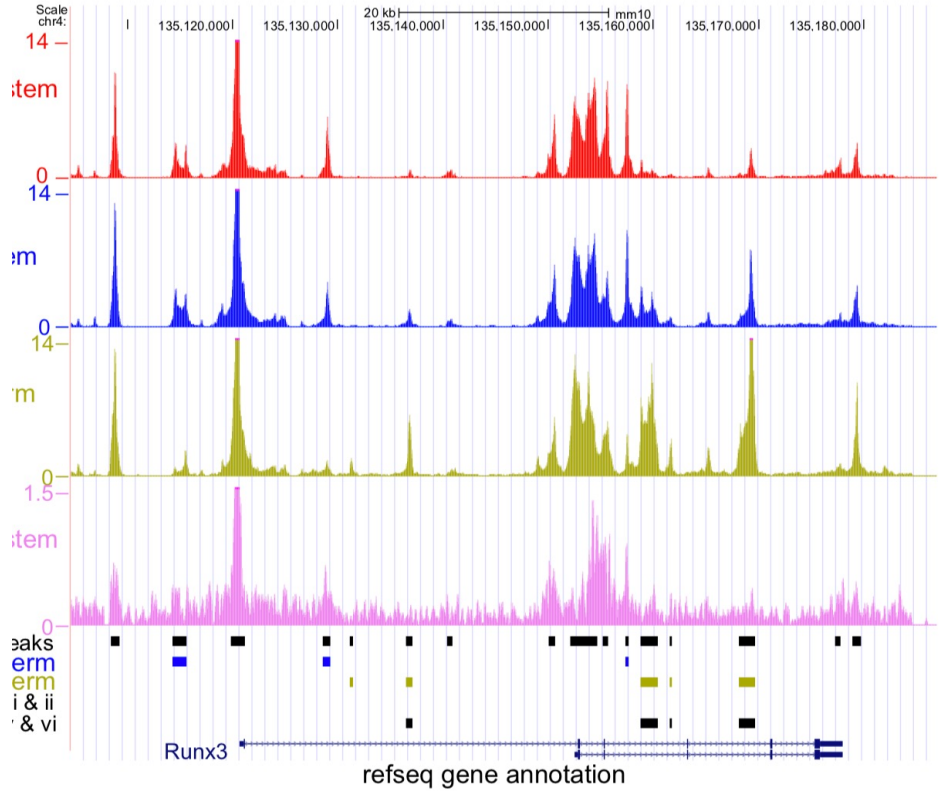
- Support site - for questions about Bioconductor packages
- Bioc-devel mailing list - for package developers

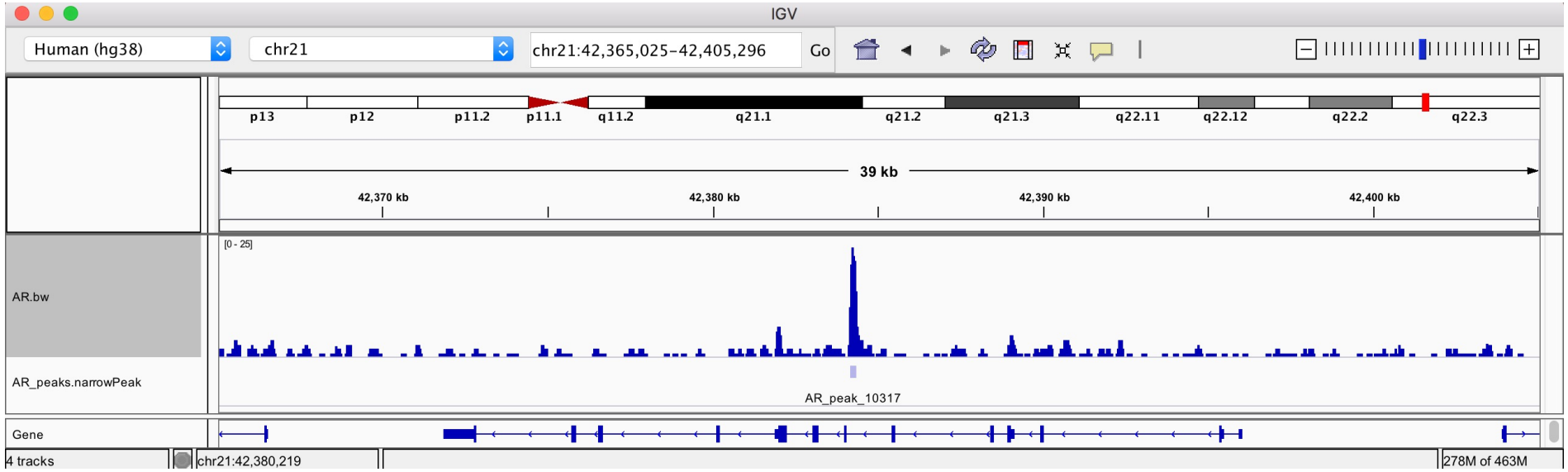
ChIP-seq: Downstream analyses

- Data visualization
  - UCSC genome browser: http://genome.ucsc.edu/
  - WashU epigenome browser: http://epigenomegateway.wustl.edu/
  - IGV: http://software.broadinstitute.org/software/igv/
- Integration with gene expression
  - BETA: http://cistrome.org/BETA/
- Integration with other epigenomic data
  - BART: http://bartweb.org/
  - MARGE: http://cistrome.org/MARGE/
  - GREAT: http://great.stanford.edu
  - ENCODE SCREEN: http://screen.umassmed.edu/

Data Visualization

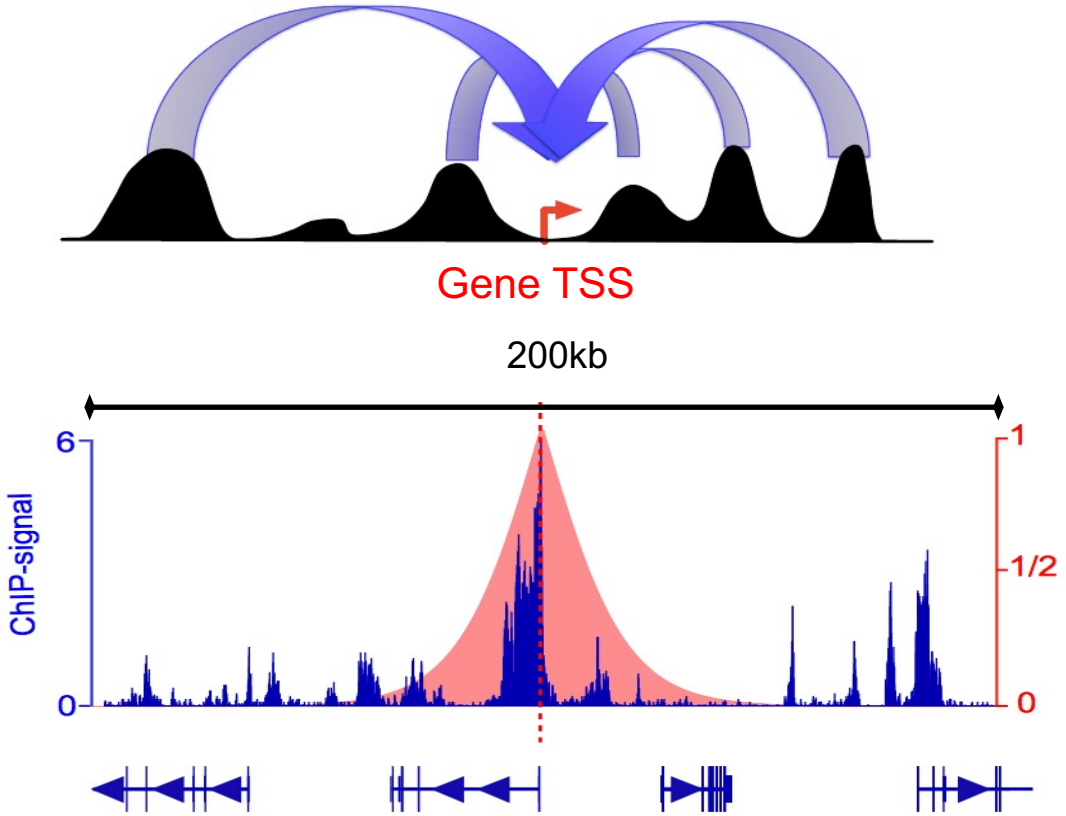
- bedGraph to bigWig
- macs2 output data
- IGV, UCSC, etc.





BETA

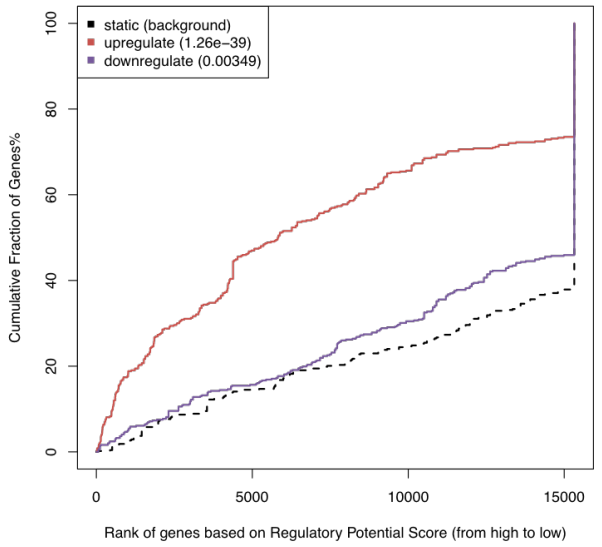
- Binding Expression Target Analysis (*Wang et al. Nat Protoc.* 2013)
- defines Regulatory Potential on each gene



$$P_i = \sum_{|j|<10^5} W_j Z_j$$

$$W_j = \frac{2\exp(-\alpha|j|)}{1+\exp(-\alpha|j|)}$$

$$\alpha = \frac{\log 3}{10^4}$$



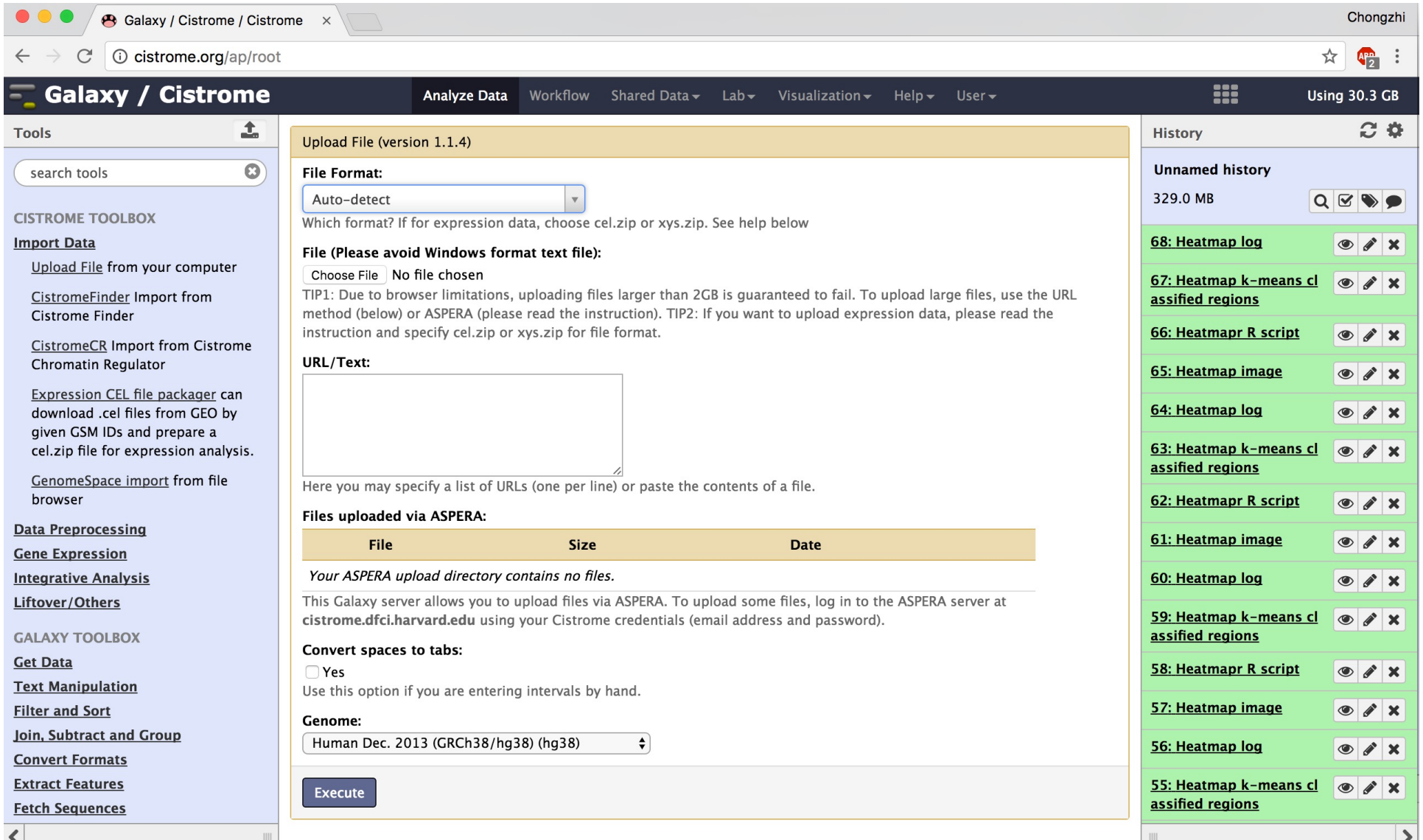
Wang, et al. *Nat Protoc* 2013
Wang, Zang et al. *Genome Res* 2016
Qin et al. *Genome Bio* 2020

Galaxy: web-interface analysis platform

- https://usegalaxy.org/

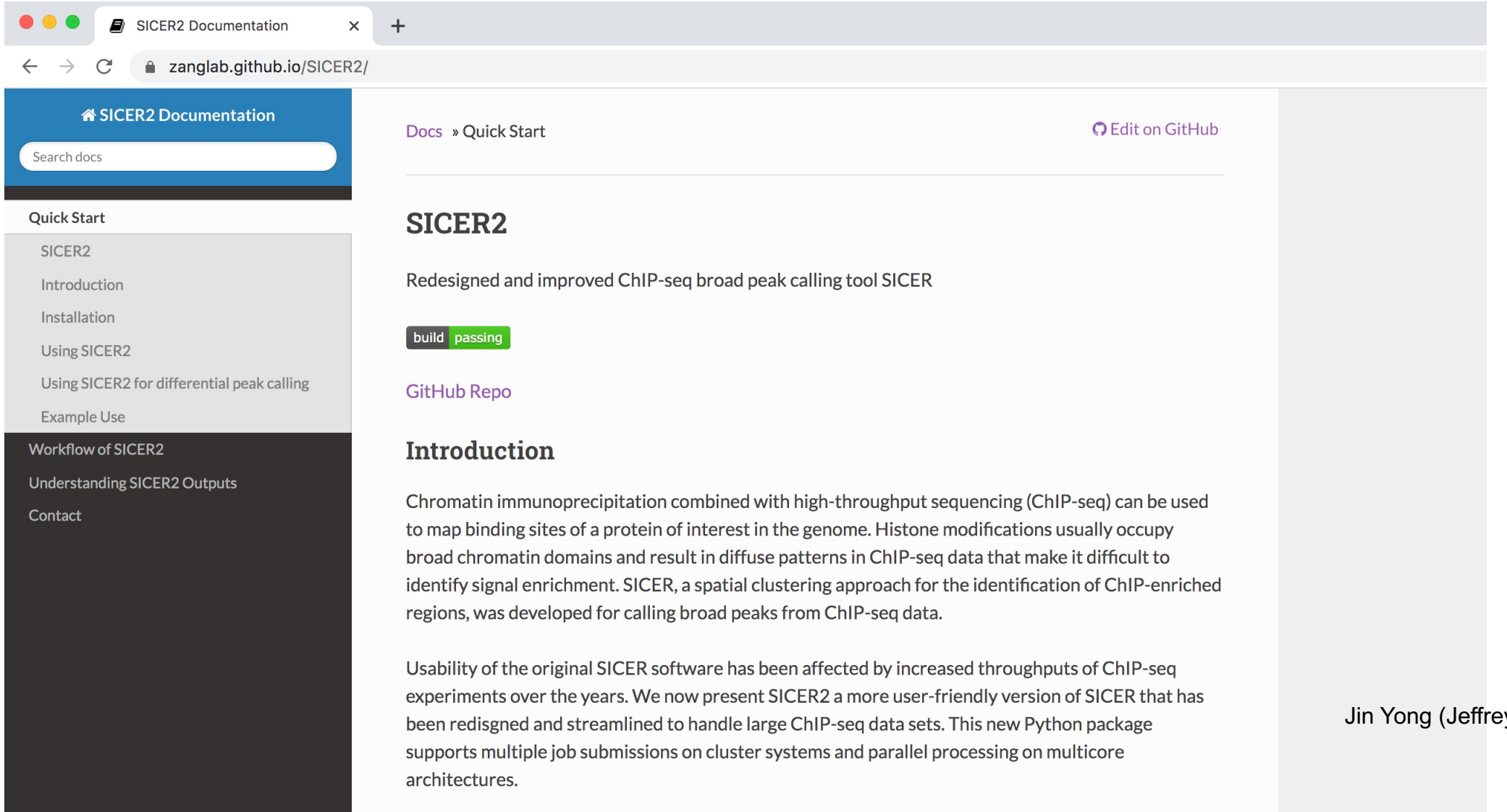
Run MACS on Cistrome, a Galaxy-based platform

- http://cistrome.org/ap/



SICER2

- https://zanglab.github.io/SICER2/

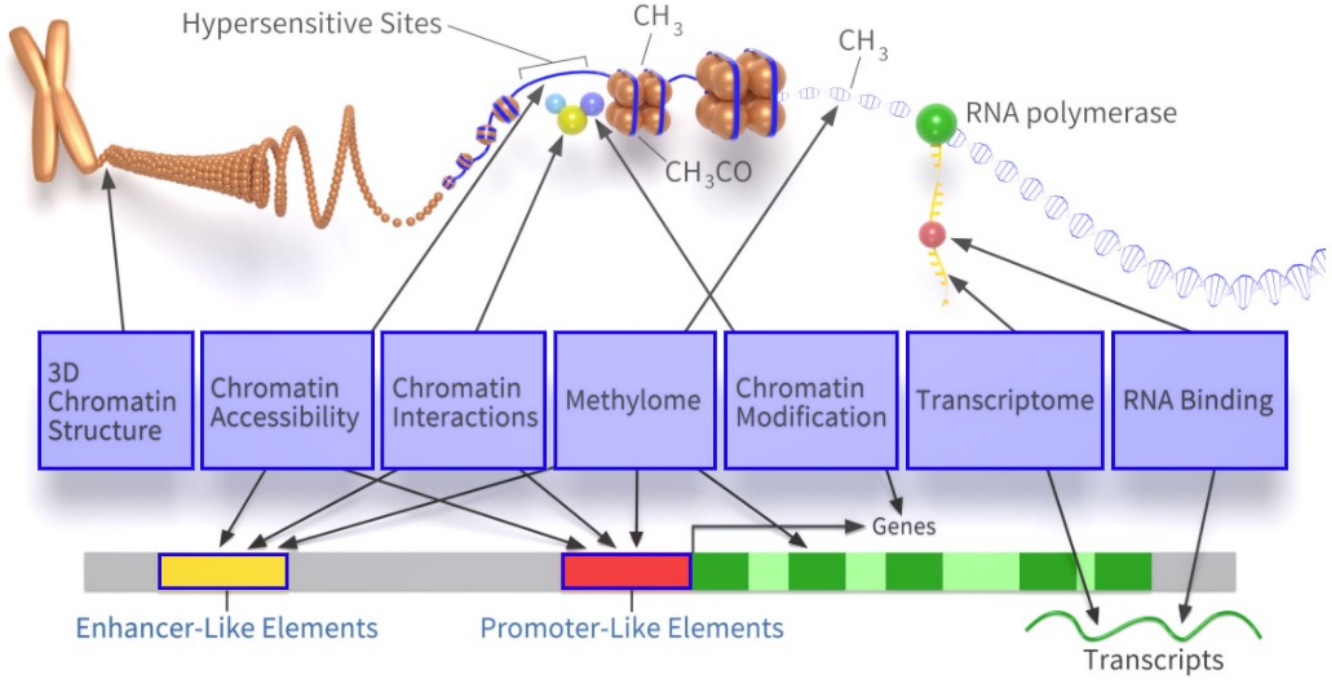


https://www.encodeproject.org/

ENCODE

ENCODE Data Encyclopedia Materials & Methods Help New » Search... Sign in / Create account

ENCODE: Encyclopedia of DNA Elements



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Search ENCODE portal

ENCODE | Functional Characterization Experiments

About ENCODE Encyclopedia | candidate Cis-Regulatory Elements

Search for candidate Cis-Regulatory Elements

Hosted by SCREEN

Human GRCh38 | Mouse mm10

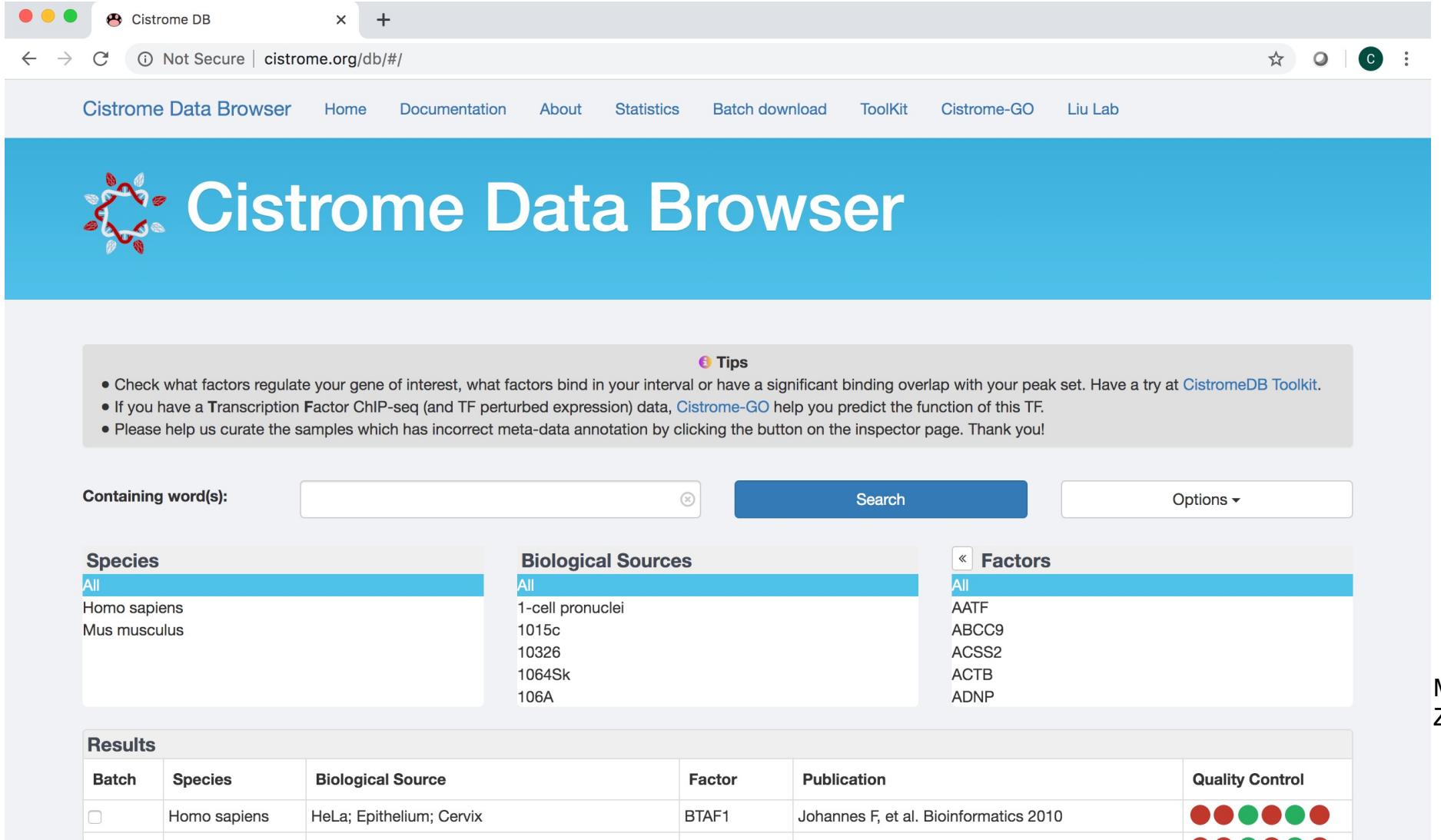
Visit hg19 site

Based on an image by Darryl Leja (NHGRI), Ian Dunham (EBI), Michael Pazin (NHGRI)

HUMAN | MOUSE | WORM | FLY

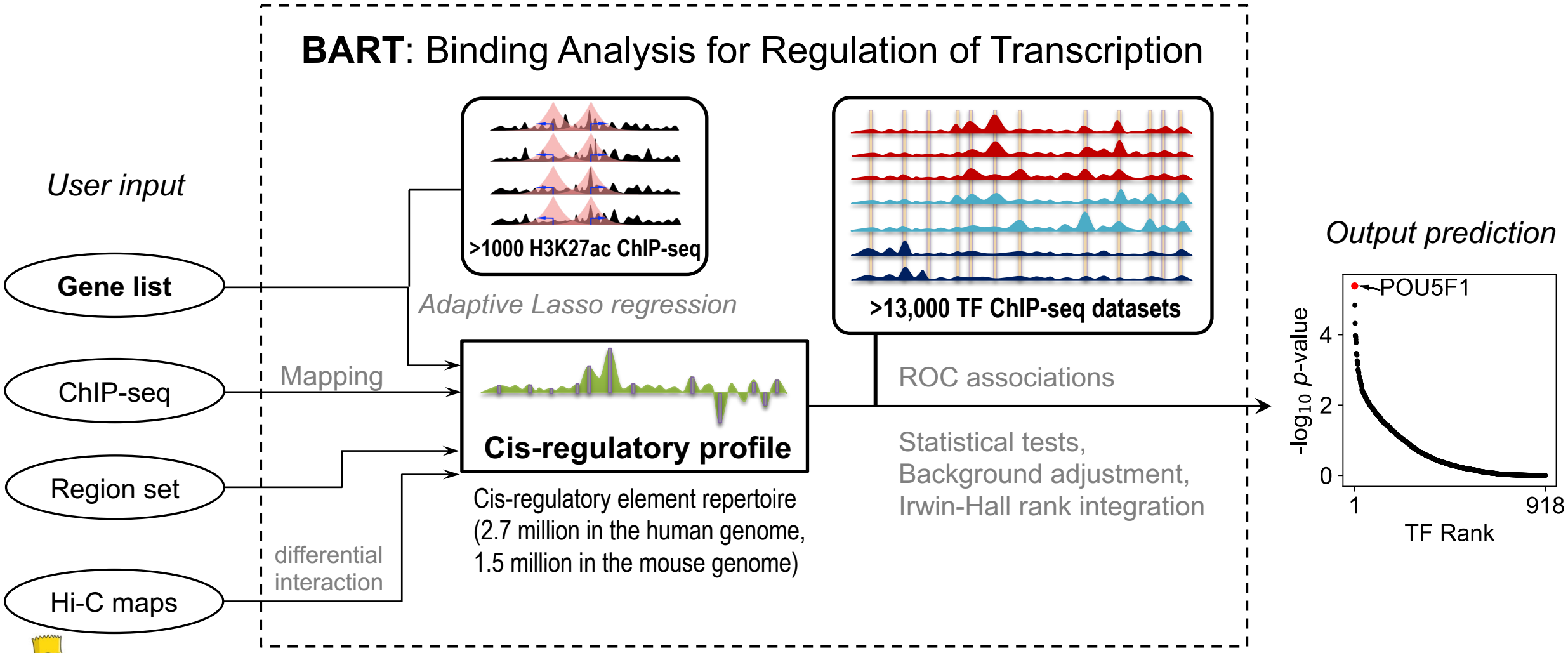
Cistrome Data Browser

http://cistrome.org/db/



Mei *et al. Nucleic Acids Res*. 2017
Zheng *et al. Nucleic Acids Res*. 2018

BART web: inferring transcriptional regulators from a variety of inputs



http://bartweb.org

Wang *et al., Bioinformatics* 2018
Wang *et al.*, *Bioinformatics* 2021
Ma, Wang *et al., NAR Genomics & Bioinformatics* 2021

Limitations of NGS for epigenetics research

- Dependent on assays (e.g., antibody availability and quality for ChIP-seq)
- Semi-quantitative: does not detect global change
- Needs many cells – difficult for clinical samples
- Cellular heterogeneity

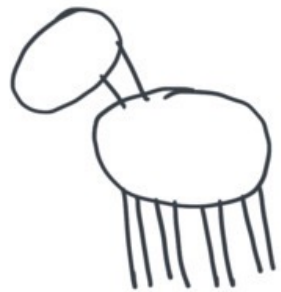
Take-home messages

- Why am I learning these if I am not a bioinformatician?
  - Help improve experimental design
  - Quality control
  - Better interpret the experimental data
  - Take advantage of existing tools and data resources

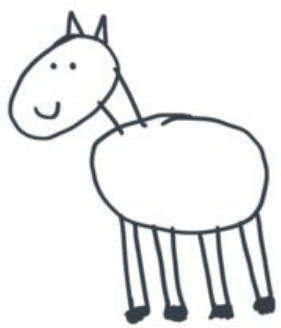
HOW TO:
DRAW A HORSE

BY VAN OKTOP

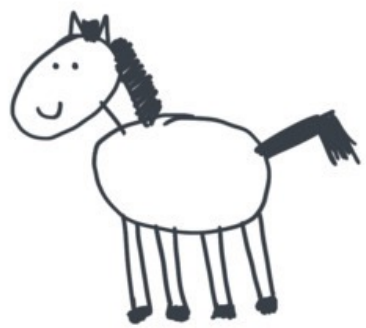
(1) DRAW 2 CIRCLES



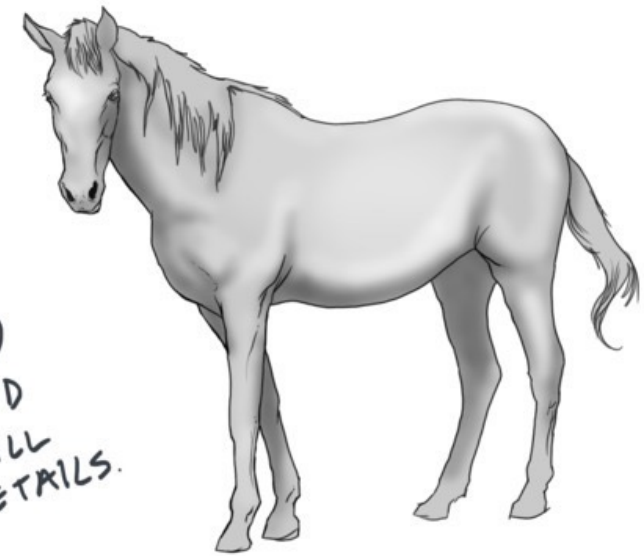
(2) DRAW THE LEGS



(3) DRAW THE FACE



(4) DRAW THE HAIR



(5) ADD SMALL DETAILS.

Thank you very much!

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