Peak-calling for ChIP-seq

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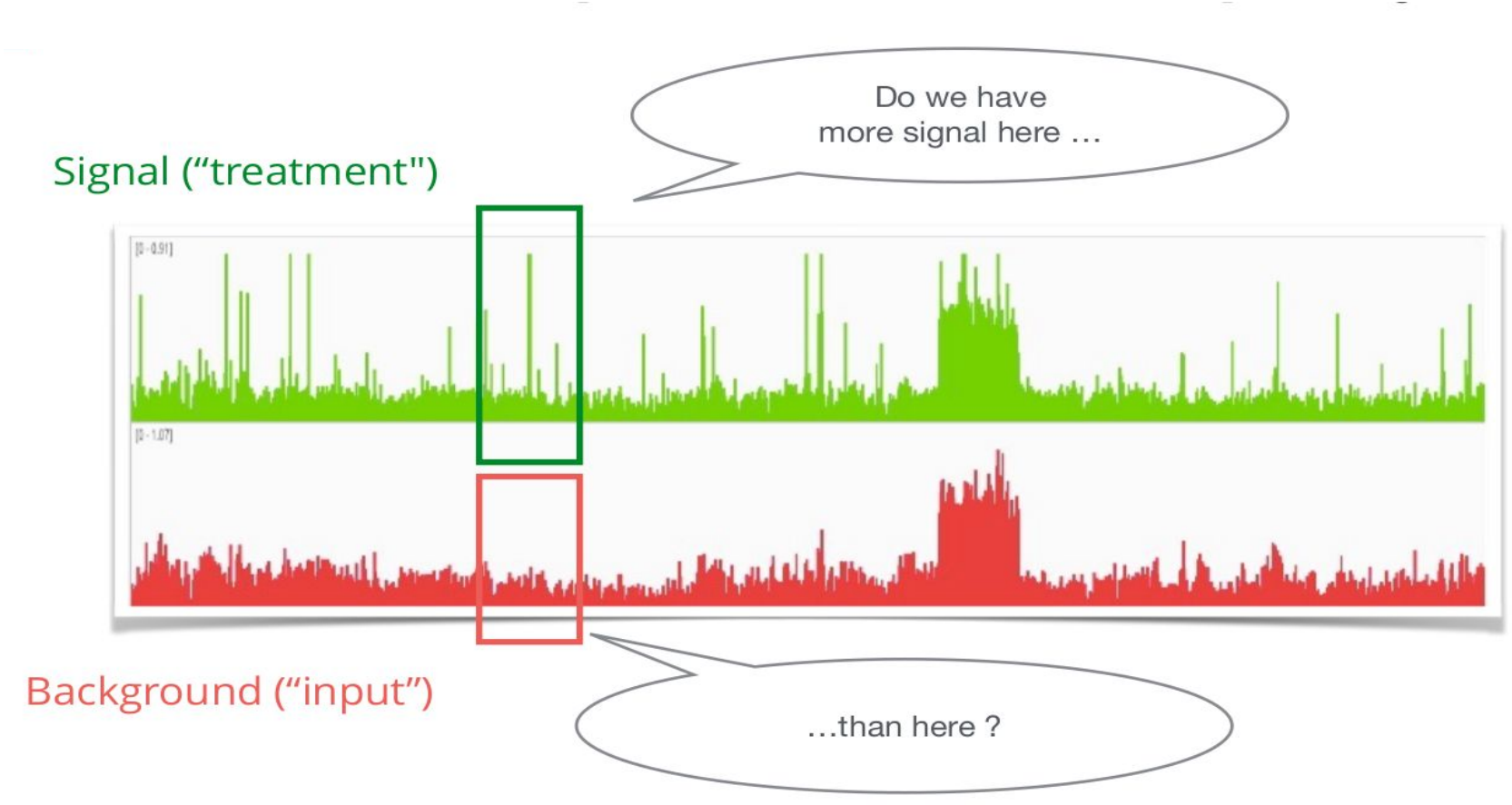
Overview

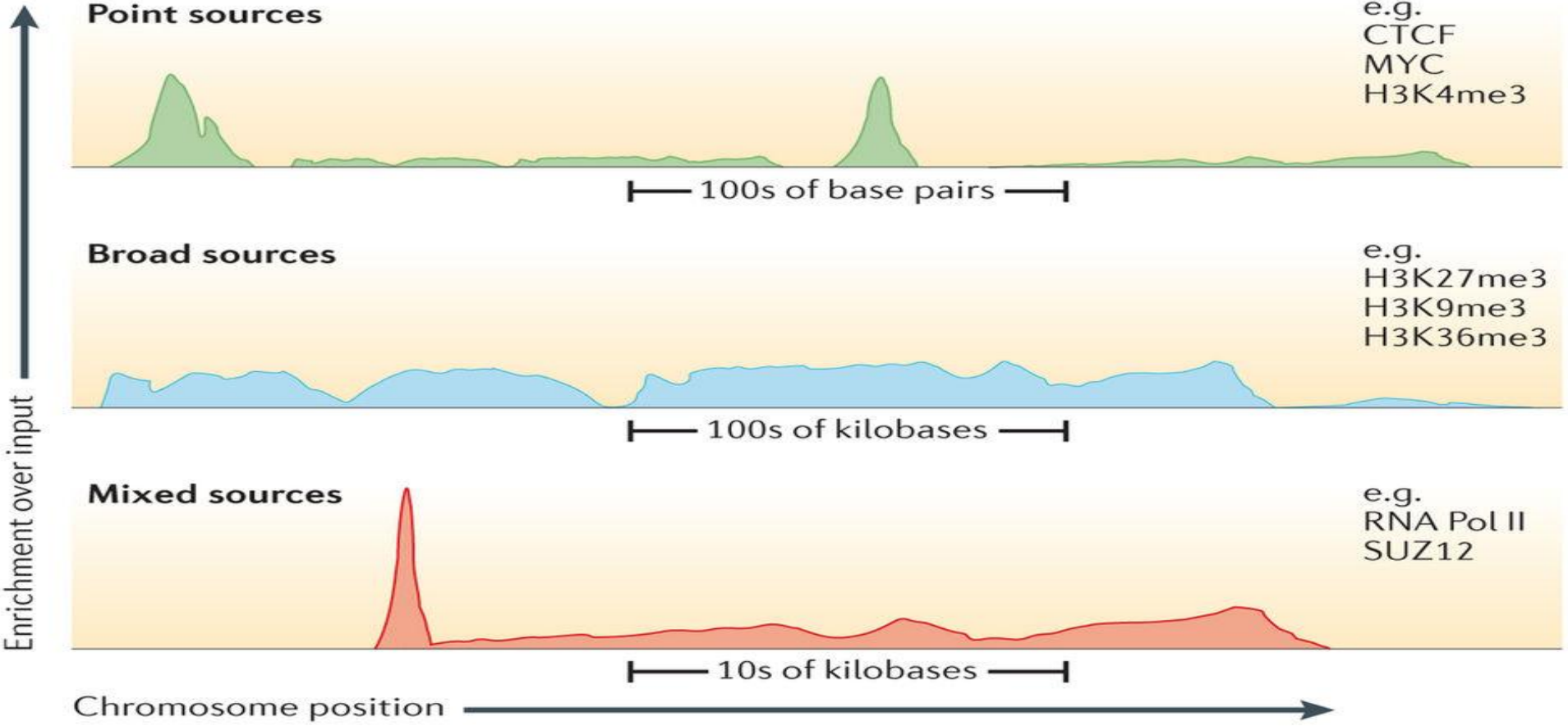
- Peak types: Narrow and Broad Peaks, Domains
- Encode Project
- Software packages
- Practical and Statistical aspects (Strand cross correlation, IDR and other QC measures)
- Duplicate filtering and Fragment length calculation
- MACS2 peak calling
- A brief look at the MACS2 settings and methodology

Peaks: Signal to Noise



modified from Carl Herrmann

Peak Shapes



Sims et al., 2014 Nat Rev Genet.

Nature Reviews | Genetics

Narrow, Broad and Mixed Peaks

- Different data types have different peak shapes. Use appropriate peak callers or domain detectors. Same TF may have different peak shapes reflecting differences in biological conditions. Replicates should have similar binding patterns.
- Most TF peaks are narrow, with particularly sharp peaks from ChIP-exo data.
- ChIP-seq peaks from epigenomic data can be narrow, broad or gapped. Histone marks such as $H3K9me^3$ or $H3K27me^3$ are broad while others such as $H3K4me^3$ and proteins such as CTCF are narrow. Other DNA binding proteins such as HP1 , Lamins (Lamin A or B), HMGA etc. form broad peaks or domains.
- PolII peaks can be narrow or broad depending on whether its detecting transcription initiation at the TSS or propagation along the gene body.

Broad peak and Domain callers

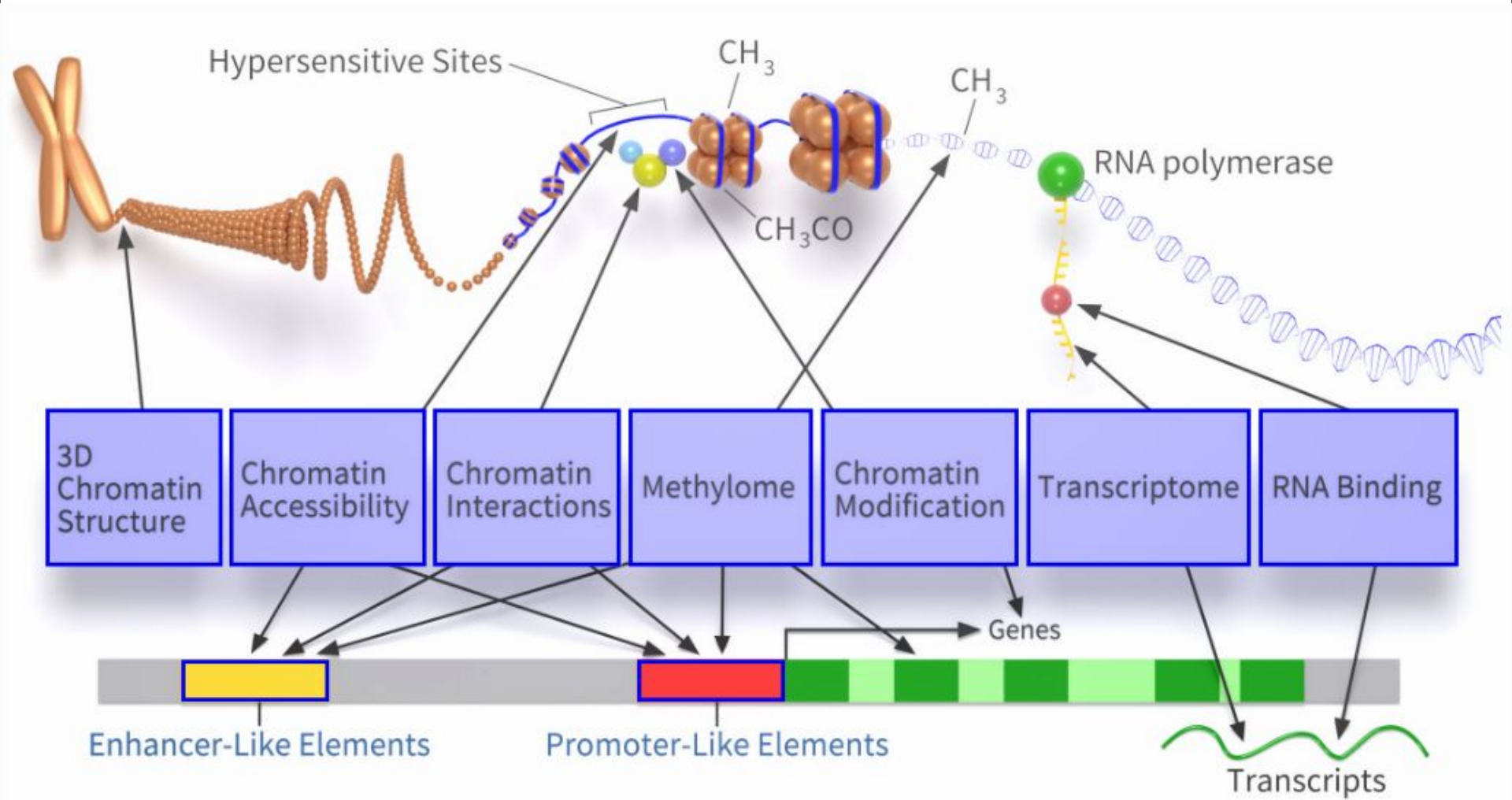
- ***MACS2*** default setting calls narrow peaks

  For broad peaks: *macs2 callpeak --broad*

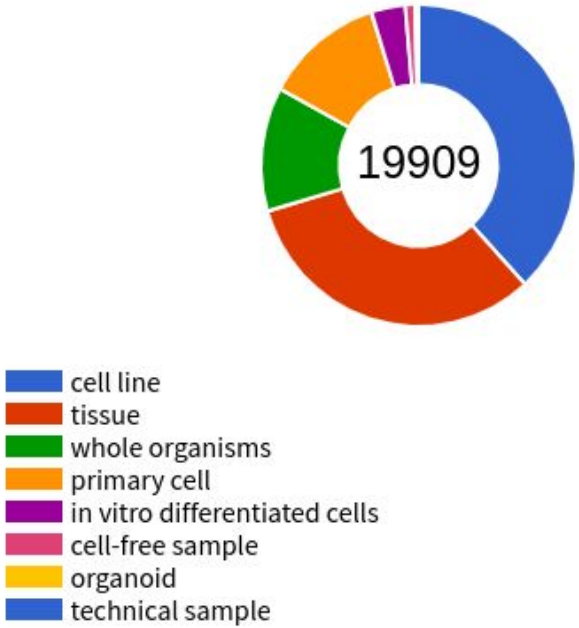
- ***Epic*****:** Useful for finding medium or diffusely enriched domains in chip-seq data. Epic is an improvement over the original SICER, by being faster, more memory efficient, multi core, and significantly easier to install and use.

- Others: ***Enriched Domain Detector (EDD)***, ***RSEG***, ***BroadPeak***, ***PeakRanger (CCAT)***

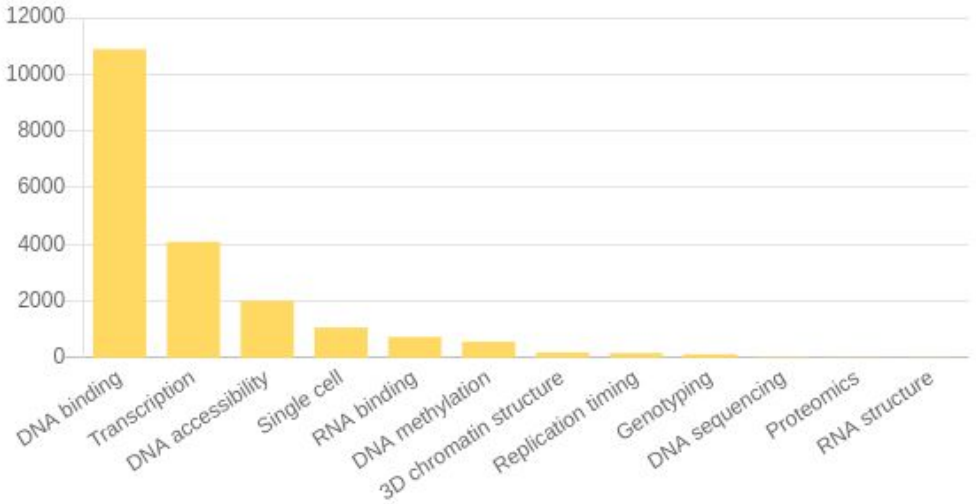
ENCODE: Encyclopedia of DNA Elements



Biosample Type



Assay Categories



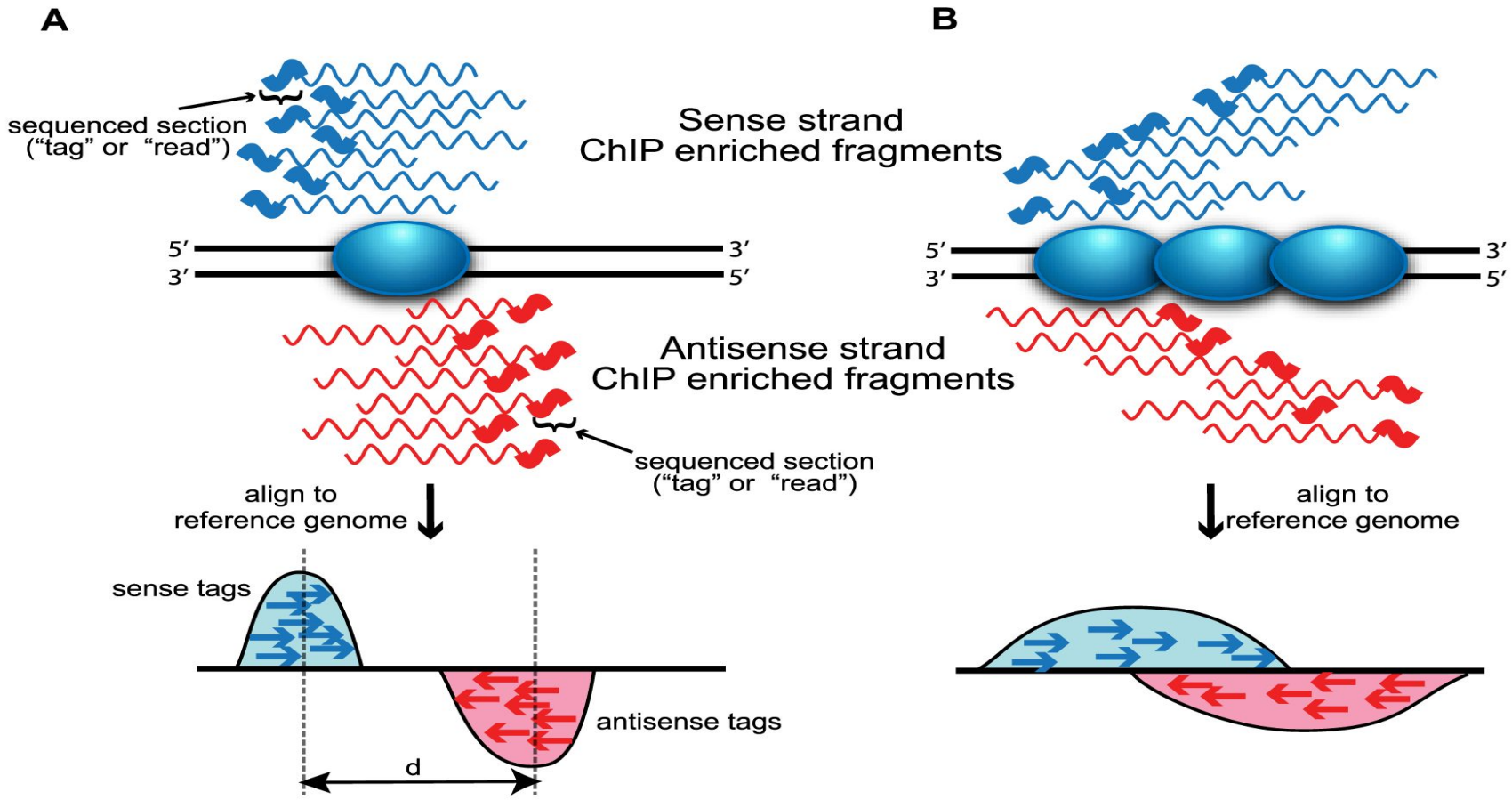
Encode Quality Metrics

Assay	Cell	Target	Treatment	Identifier	N_uniq map reads	MACS FDR 0.01	Self Cons IDR 0.02	Rep Cons IDR 0.01	SPOT	PBC	NSC	RSC	Under seq	Diff rep	Manual low S/N	Auto low S/N
TF-ChIP-seq	A549	CTCF	DEX_100nM	wgEncodeHaibTfbsA549CtcfPcr1xDexaAlnRep1	24,281,189	38,537	45,841	30,324	0.2361	0.71	2.79	2.19	0	0	0	0
TF-ChIP-seq	A549	CTCF	DEX_100nM	wgEncodeHaibTfbsA549CtcfPcr1xDexaAlnRep2	15,453,361	96,884	39,091	30,324	0.1249	0.41	1.84	2.31	0	1	0	0
TF-ChIP-seq	A549	GR	DEX_100nM	wgEncodeHaibTfbsA549GrPcr2xDexaAlnRep1	16,608,102	9,921	12,613	8,283	0.0754	0.91	1.38	1.21	0	1	0	0
TF-ChIP-seq	A549	GR	DEX_100nM	wgEncodeHaibTfbsA549GrPcr2xDexaAlnRep2	28,467,922	8,683	12,880	8,283	0.0723	0.44	1.42	1	0	0	0	0
TF-ChIP-seq	A549	POL2	DEX_100nM	wgEncodeHaibTfbsA549Pol2Pcr2xDexaAlnRep1	19,005,470	12,689	24,395	21,463	0.6166	0.86	2.99	1.32	0	0	0	0
TF-ChIP-seq	A549	POL2	DEX_100nM	wgEncodeHaibTfbsA549Pol2Pcr2xDexaAlnRep2	23,115,884	14,816	28,503	21,463	0.5388	0.86	2.81	1.12	0	0	0	0
TF-ChIP-seq	A549	USF1	DEX_100nM	wgEncodeHaibTfbsA549Usf1Pcr1xDexaAlnRep1	22,289,881	2,631	16,330	8,917	0.0791	0.87	1.28	1.86	0	0	0	0
TF-ChIP-seq	A549	USF1	DEX_100nM	wgEncodeHaibTfbsA549Usf1Pcr1xDexaAlnRep2	12,364,820	3,028	7,659	8,917	0.0517	0.82	1.44	1.9	0	0	0	0
TF-ChIP-seq	A549	GR	DEX_500pM	wgEncodeHaibTfbsA549GrPcr1xDexdAlnRep1	19,646,503	25,233	1,312	1,226	0.0105	0.96	1.05	0.56	0	0	1	1
TF-ChIP-seq	A549	GR	DEX_500pM	wgEncodeHaibTfbsA549GrPcr1xDexdAlnRep2	15,095,316	123,828	1,218	1,226	0.0109	0.94	1.06	0.5	0	0	1	1
TF-ChIP-seq	A549	GR	DEX_50nM	wgEncodeHaibTfbsA549GrPcr1xDexbAlnRep1	19,291,260	57,488	23,821	25,096	0.1289	0.96	1.55	1.42	0	0	0	0
TF-ChIP-seq	A549	GR	DEX_50nM	wgEncodeHaibTfbsA549GrPcr1xDexbAlnRep2	16,754,796	71,917	22,601	25,096	0.1426	0.95	1.64	1.61	0	0	0	0
TF-ChIP-seq	A549	GR	DEX_5nM	wgEncodeHaibTfbsA549GrPcr1xDexcAlnRep1	20,120,740	19,331	8,573	10,348	0.0343	0.98	1.10	0.89	0	1	1	0
TF-ChIP-seq	A549	GR	DEX_5nM	wgEncodeHaibTfbsA549GrPcr1xDexcAlnRep2	20,559,786	31,539	13,796	10,348	0.0641	0.96	1.23	1.17	0	0	0	0
TF-ChIP-seq	A549	CTCF	EtOH_0.02p	wgEncodeHaibTfbsA549CtcfPcr1xEtoh02AlnRep1	22,672,467	31,983	37,735	33,511	0.1601	0.75	1.78	2.67	0	0	0	0
TF-ChIP-seq	A549	CTCF	EtOH_0.02p	wgEncodeHaibTfbsA549CtcfPcr1xEtoh02AlnRep2	14,351,615	236,390	49,814	33,511	0.2040	0.42	3.21	2.55	0	0	0	0
TF-ChIP-seq	A549	POL2	EtOH_0.02p	wgEncodeHaibTfbsA549Pol2Pcr2xEtoh02AlnRep1	17,136,347	17,929	29,121	28,130	0.5602	0.9	2.89	1.19	0	0	0	0
TF-ChIP-seq	A549	POL2	EtOH_0.02p	wgEncodeHaibTfbsA549Pol2Pcr2xEtoh02AlnRep2	19,201,309	16,879	34,156	28,130	0.5687	0.82	3.09	1.12	0	0	0	0
TF-ChIP-seq	A549	USF1	EtOH_0.02p	wgEncodeHaibTfbsA549Usf1Pcr1xEtoh02AlnRep1	16,241,779	7,936	11,349	10,368	0.0648	0.95	1.38	2.02	0	0	0	0
TF-ChIP-seq	A549	USF1	EtOH_0.02p	wgEncodeHaibTfbsA549Usf1Pcr1xEtoh02AlnRep2	13,242,129	11,812	11,204	10,368	0.0793	0.85	1.72	1.99	0	0	0	0
TF-ChIP-seq	AG04449	CTCF	None	wgEncodeUwTfbsAg04449CtcfStdAlnRep1	9,952,444	97,323	62,334	44,965	0.5513	0.85	11.97	2.11	0	0	0	0
TF-ChIP-seq	AG04449	CTCF	None	wgEncodeUwTfbsAg04449CtcfStdAlnRep2	23,572,200	42,477	42,096	44,965	0.2187	0.94	2.68	1.61	0	0	0	0
TF-ChIP-seq	AG04450	CTCF	None	wgEncodeUwTfbsAg04450CtcfStdAlnRep1	21,170,101	44,837	43,626		0.2450	0.9	2.62	1.73	0	0	0	0
TF-ChIP-seq	AG09309	CTCF	None	wgEncodeUwTfbsAg09309CtcfStdAlnRep1	14,311,099	37,977	35,062	35,451	0.3278	0.89	3.93	1.8	0	0	0	0
TF-ChIP-seq	AG09309	CTCF	None	wgEncodeUwTfbsAg09309CtcfStdAlnRep2	10,263,622	34,845	31,992	35,451	0.1768	0.95	2.31	1.52	0	0	0	0
TF-ChIP-seq	AG09319	CTCF	None	wgEncodeUwTfbsAg09319CtcfStdAlnRep1	22,451,182	53,232	42,690	34,945	0.3807	0.8	4.32	1.67	0	0	0	0
TF-ChIP-seq	AG09319	CTCF	None	wgEncodeUwTfbsAg09319CtcfStdAlnRep2	25,700,109	45,377	38,947	34,945	0.2775	0.87	2.97	1.73	0	0	0	0
TF-ChIP-seq	AG10803	CTCF	None	wgEncodeUwTfbsAg10803CtcfStdAlnRep1	26,964,677	39,701	38,287	39,892	0.2254	0.88	2.36	1.63	0	0	0	0

Peak Calling Software

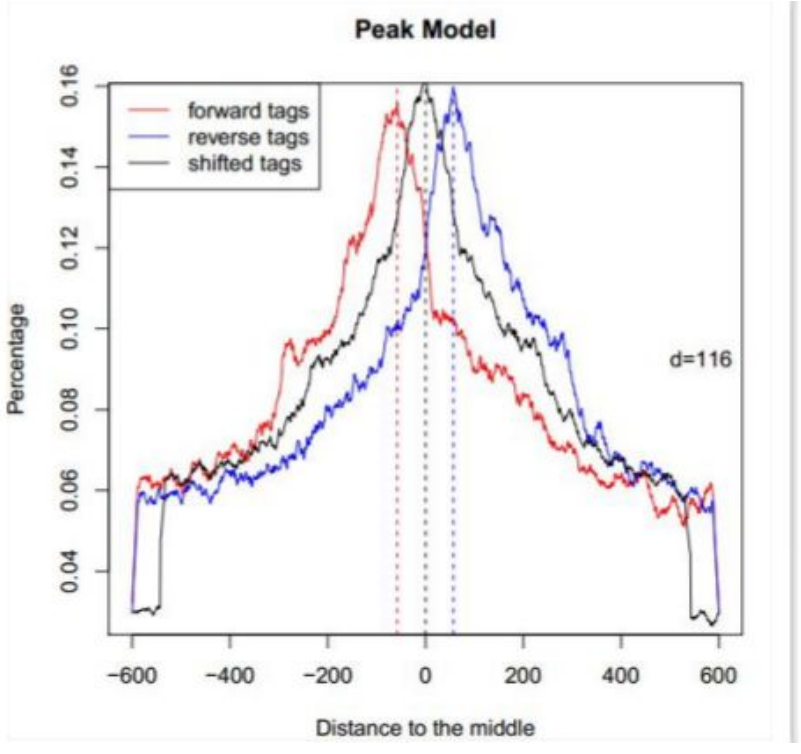
MACS2 (MACS3 soon)	Most widely used peak caller. Can detect narrow and broad peaks.
Epic (SICER)	Specialised for broad peaks
BayesPeak	R/Bioconductor
Jmosaics	Detects enriched regions jointly from replicates
T-PIC	Shape based
EDD	Detects megabase domain enrichment
GEM	Peak calling and motif discovery for ChIP-seq and ChIP-exo
SPP	Fragment length computation and saturation analysis to determine if read depth is adequate.

Strand dependent bimodality



Reads to Peaks

- +ive and -ive strand reads do not represent true binding sites
- Fragment length *d* can be detected experimentally or estimated from strand asymmetry in data
- Reads from both strands can be **extended** to the length of *d* **OR**
- Reads can be **shifted** towards 3' by *d*/2



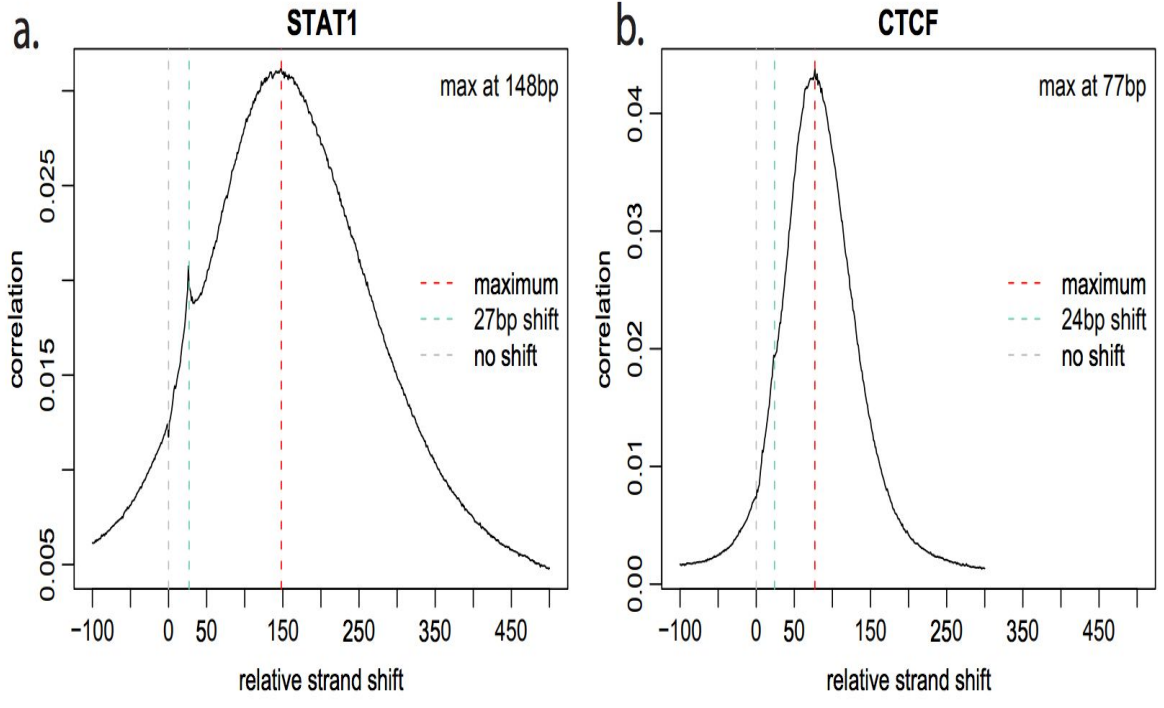
Peak Quality Measures

Relative strand cross-correlation

Strand cross-correlation is computed as the Pearson's linear correlation between the minus strand and the plus strand, after shifting minus strand by k base pairs. The result is a cross-correlation value for each shift value, and they can be plotted against each other to generate a cross-correlation plot as shown below.

RSC=(fragment length CC-background CC)/(phantom peak CC-background CC)

- high enrichment: RSC values > 1
- low signal-to-noise: RSC values < 0.8
- minimum possible RSC value: 0 (no enrichment)



https://github.com/kundajelab/phantompeakqualtools

Quality Measures

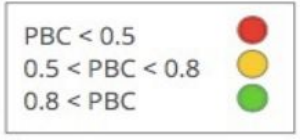
- Fraction of reads in peaks (FRiP) is dependant on data type.

$$FRiP = \frac{reads \in peaks}{total\ reads}$$

FRiP can be calculated with deepTools2

- PCR Bottleneck Coefficient (PBC) is a measure of library complexity

$$PBC = \frac{N_1}{N_2}$$



N1= Non redundant, uniquely mapping reads
N2= Uniquely mapping reads

Preseq and preseqR for determining library complexity
Daley et al., 2013, Nat. Methods

IDR: Irreproducible Discovery Rate

- If two replicates measure the same underlying biology, the most **significant peaks** which are likely to be genuine signals, are expected to have **high consistency between replicates**. Peaks with low significance, which are more likely to be noise, are expected to have low consistency.

- IDR **measures consistency between replicates** in high-throughput experiments. The IDR method compare a pair of ranked lists of identifications (such as ChIP-seq peaks). These ranked lists should not be pre-thresholded, i.e they should provide identifications across the entire spectrum of high confidence/enrichment (signal) and low confidence/enrichment (noise).

- The method uses reproducibility in score rankings between peaks in each replicate to determine an optimal cutoff for significance. The IDR method then fits the bivariate rank distributions over the replicates in order to separate signal from noise based on a defined confidence of rank consistency and reproducibility of identifications.

  software: https://github.com/nboley/idr

Duplicates Removal

- Duplicates are reads or pairs of reads that have identical or near-identical sequences (due to sequencing errors) and map to the same genomic position and strand.
- During library preparation, ChIP DNA undergoes an PCR amplification step.
- Sequencing depth, low immunoprecipitation efficiency or insufficient amounts of starting material, can contribute to PCR duplicates formation.
- These types of duplicates need to be filtered out.
- Examination of read alignment (bam files) in a genome browser can help identify PCR duplicates.
- However natural duplicates arise from sequencing of independent DNA fragments derived from the same genomic locations due to sparsity of TF binding sites or regions associated with histone modification.
- These should not be removed as they are part of the true signal.

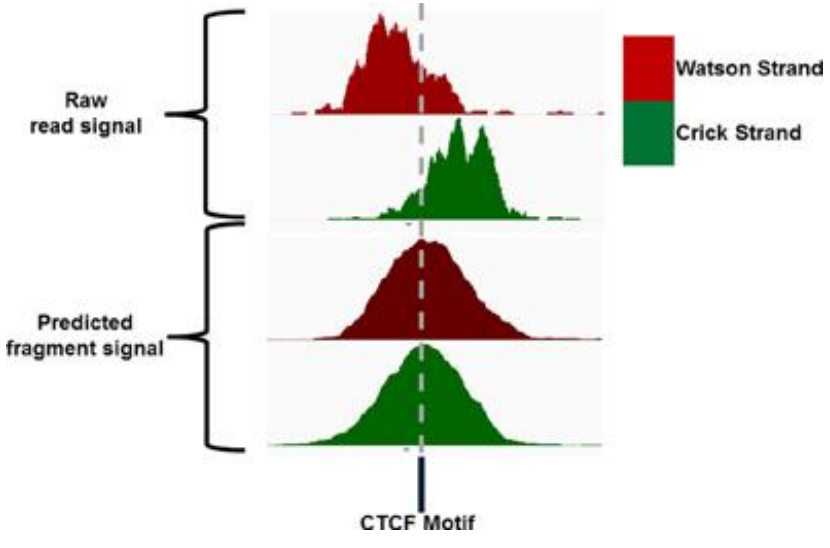
PLoS One. 2019 Apr 3;14(4):e0214723. doi: 10.1371/journal.pone.0214723. eCollection 2019.

Identification of factors associated with duplicate rate in ChIP-seq data

Shulan Tian [1], Shuxia Peng [1], Michael Kalmbach [2], Krutika S Gaonkar [1], Aditya Bhagwate [1], Wei Ding [3], Jeanette Eckel-Passow [1], Huihuang Yan [1], Susan L Slager [1]

Fragment Length Estimation

- Different sizes of chromatin fragments may work well for ChIP-PCR assays, the optimal size range of chromatin for ChIP-Seq analysis should be between 150 and 300 bp
- Sequence reads typically only represent the 5′ and 3′ end portions of DNA fragments within the library pool.
- In ChIP-seq the reconstruction of the true fragments from the available sequence reads allow for a more accurate representation of ChIP-signal across the genome and a higher resolution of epigenetic marks and DNA binding sites.



Front. Genet. | https://doi.org/10.3389/fgene.2014.00075

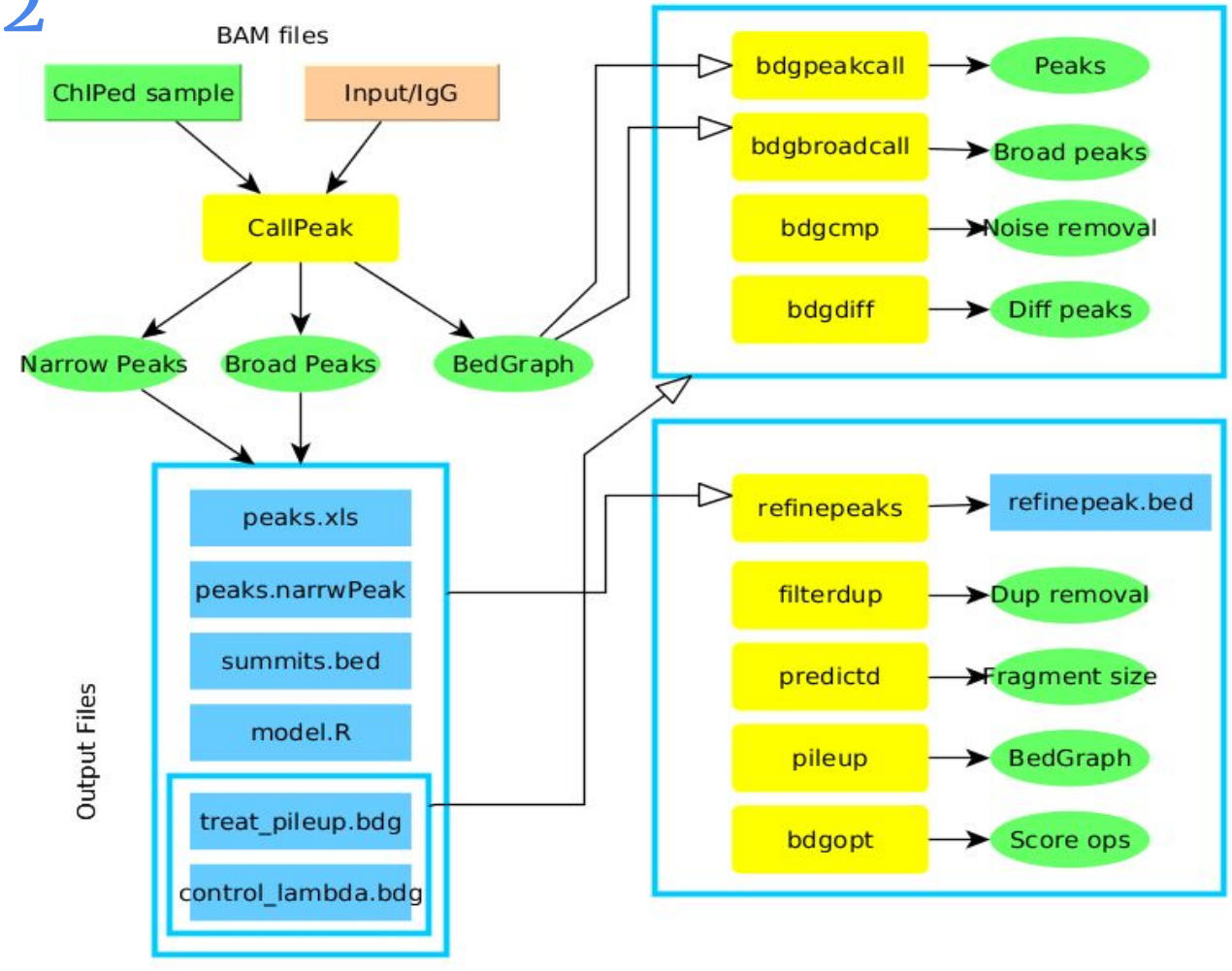
Impact of artifact removal on ChIP quality metrics in ChIP-seq and ChIP-exo data

Thomas S. Carroll[1*†], Ziwei Liang[2†], Rafik Salama[1†], Rory Stark[1] and Ines de Santiago[1*]

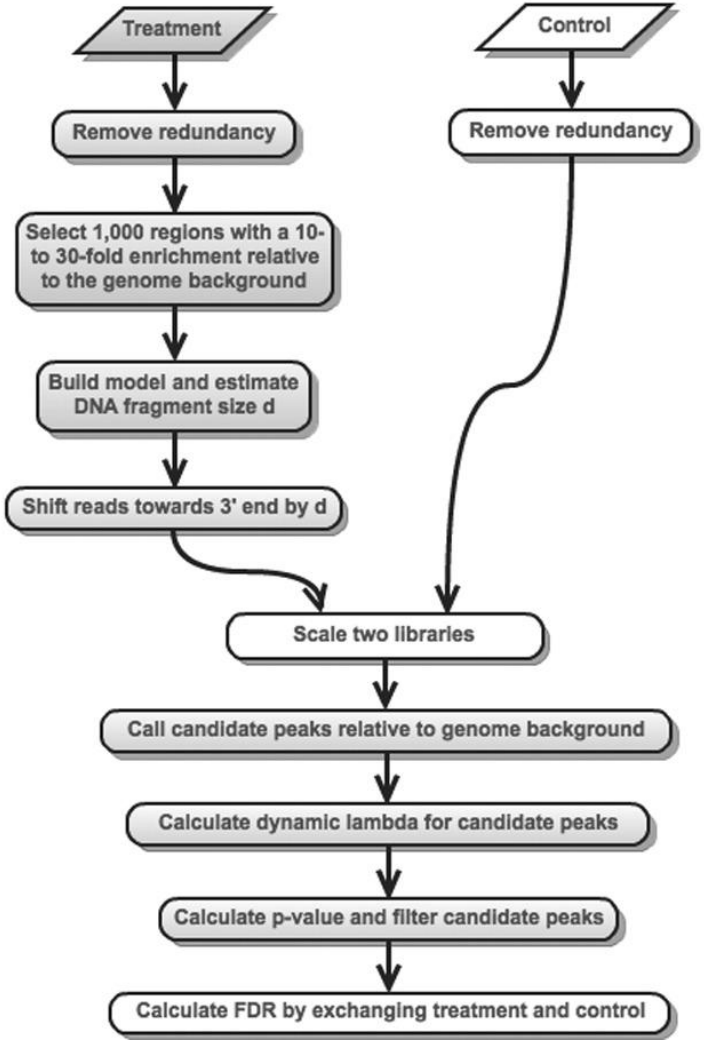
MACS2

- Most widely used peak caller.
- identifies genome-wide locations of TF binding, histone modification or NFRs from ChIP-seq or ATAC-seq data.
- Can be used without a **control** (Input -samples of sonicated chromatin OR IgG - nonspecific antibody) - Not Recommended for ChIP-seq!
- Controls **bias** due to GC content, mappability, DNA repeats or CNVs.
- Can call narrow and broad peaks.
- Many settings for optimizing results.
- MACS3 (alpha version currently available)

MACS2

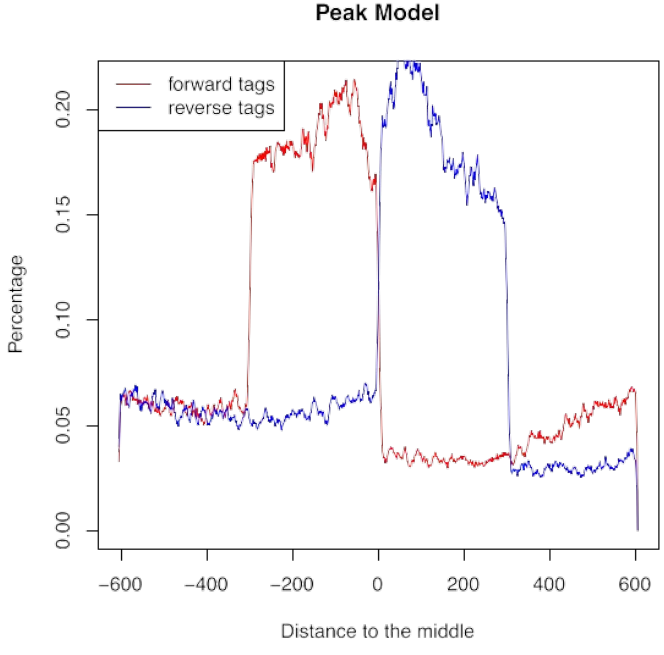

BAM files
ChIPed sample
Input/IgG
CallPeak
Narrow Peaks
Broad Peaks
BedGraph
bdgpeakcall
Peaks
bdgbroadcall
Broad peaks
bdgcmp
Noise removal
bdgdiff
Diff peaks
Output Files
peaks.xls
peaks.narrwPeak
summits.bed
model.R
treat_pileup.bdg
control_lambda.bdg
refinepeaks
refinepeak.bed
filterdup
Dup removal
predictd
Fragment size
pileup
BedGraph
bdgopt
Score ops

MACS1.4


Treatment
Control
Remove redundancy
Remove redundancy
Select 1,000 regions with a 10- to 30-fold enrichment relative to the genome background
Build model and estimate DNA fragment size d
Shift reads towards 3' end by d
Scale two libraries
Call candidate peaks relative to genome background
Calculate dynamic lambda for candidate peaks
Calculate p-value and filter candidate peaks
Calculate FDR by exchanging treatment and control

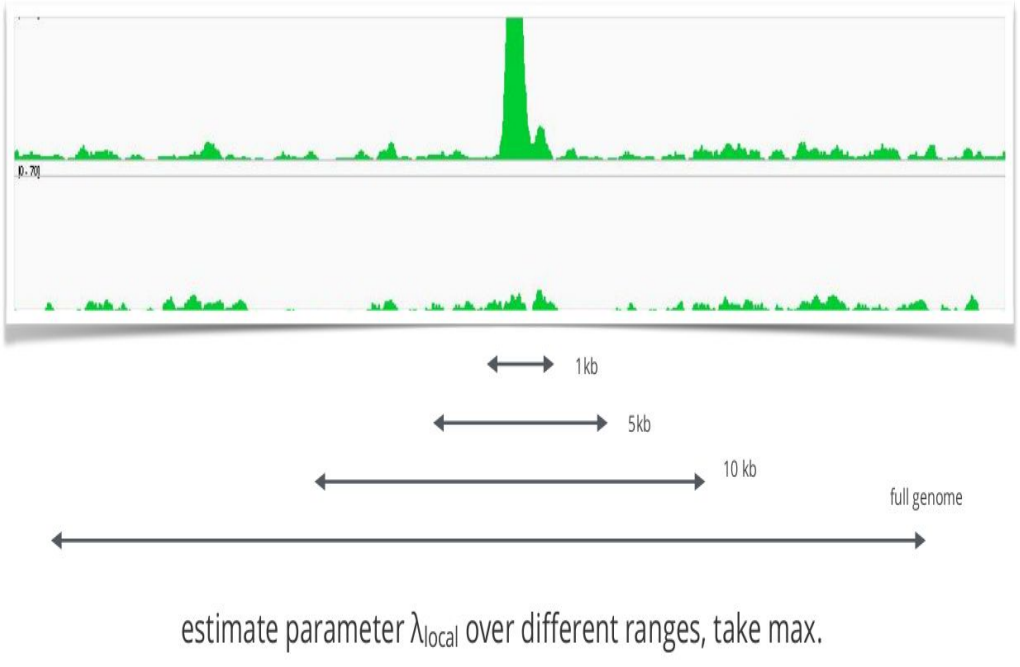
Peak calling with MACS2

- **Step 1: Estimate fragment length *d* and adjust read position**
  - Slide a window of length 2 x **bw** bandwidth (half of estimated sonication fragment size) across genome.
  - Retain windows with > **MFOLD** (*fold-enrichment of treatment / background*)
  - Compute the average +/- strand specific read-densities for these bins.



MACS2

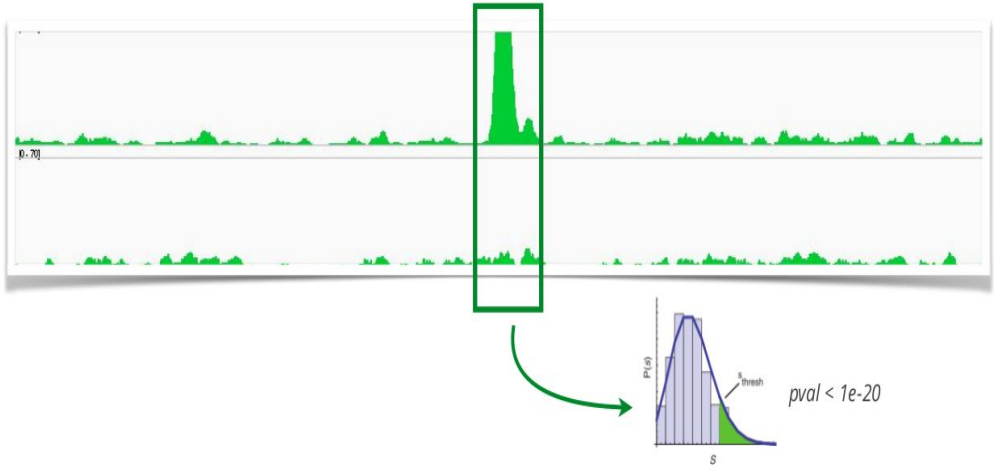
- **Step 2: Identify local noise**
  - slide a window of size 2*d across treatment and input
  - estimate λ_{local} parameter of Poisso distribution



modified from Carl Herrmann

MACS2

- **Step 3: Identify enriched (peak) regions**
  - determine regions with p-value < PVALUE
  - determine summit position within enriched regions as max density



modified from Carl Herrmann

MACS2

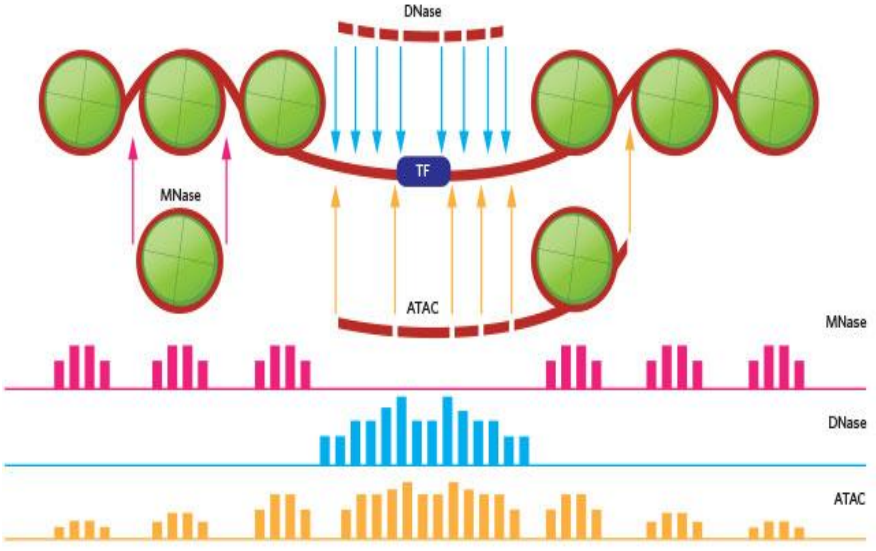
- Step 4: Estimate FDR
  - positive peaks (P-values)
  - swap treatment and input, call negative peaks (P-value)

$$FDR = \frac{\#\text{ negative peaks with pval} < p}{\#\text{ positive peaks with pval} < p}$$

modified from Carl Herrmann

MACS2 ATAC-seq settings

- If using paired end reads use "--format BAMPE" to let MACS2 pileup the whole fragments in general. If you want to focus on looking for where the 'cutting sites' are, then "--nomodel --shift -100 --extsize 200" should work.
- Since the DNA wrapped on a nucleosome is about 147bp, for single nucleosome detection use "--nomodel --shift -37 --extsize 73".



BASED ON *EPIGENETICS CHROMATIN*, 7:33, 2014.
Scientist, Volume 30 Issue 1 | January 2016

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- https://hbctraining.github.io/In-depth-NGS-Data-Analysis-Course/sessionV/lessons/CC_metrics_extra.html