ChIP-seq: Peak Calling

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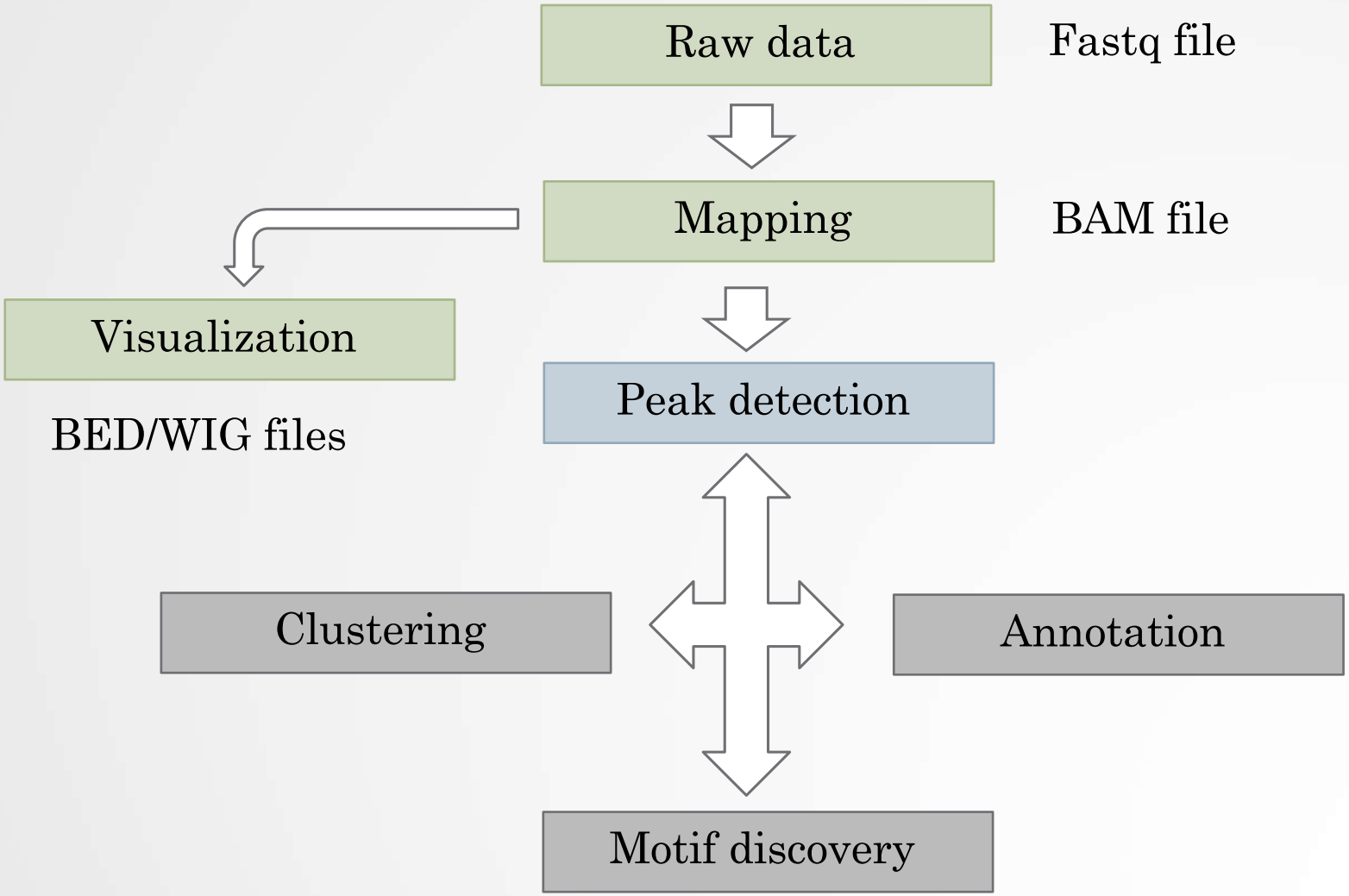
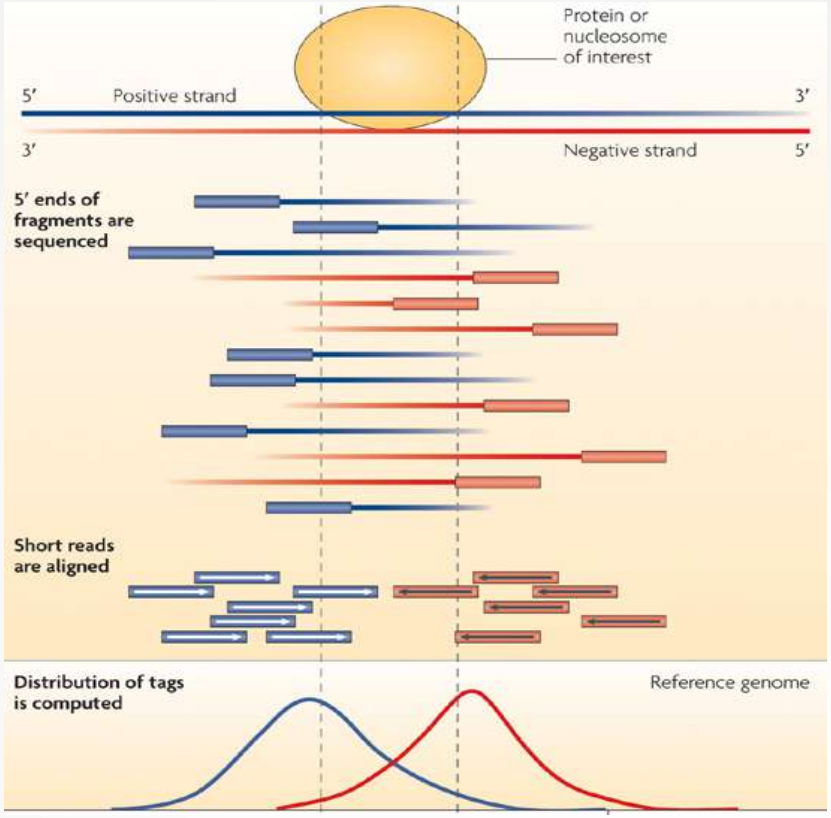
Guidelines


Raw data
Fastq file
Mapping
BAM file
Visualization
BED/WIG files
Peak detection
Clustering
Annotation
Motif discovery

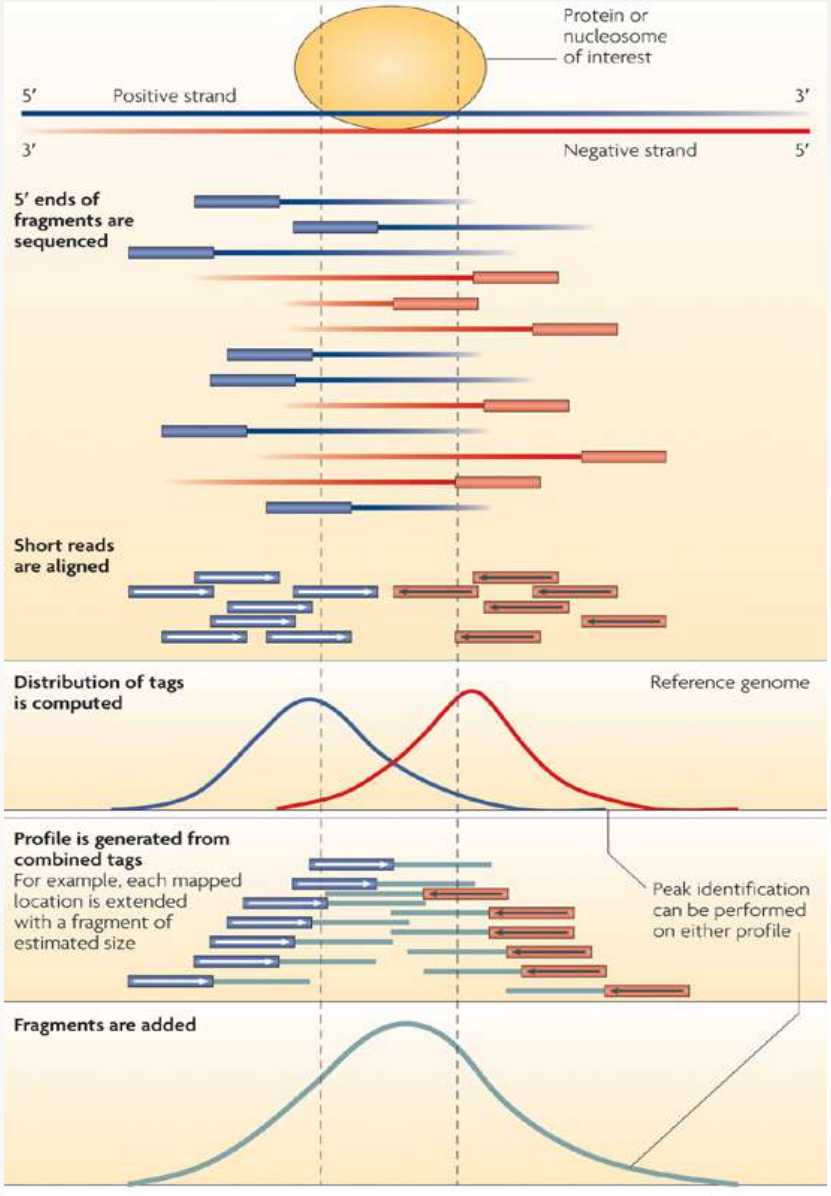
From reads to peaks

- Chip-seq peaks are a mixture of two signals:
  - + strand reads (Watson)
  - - strand reads (Cricks)
- The sequence tag density accumulates on forward and reverse strands centered around the binding site



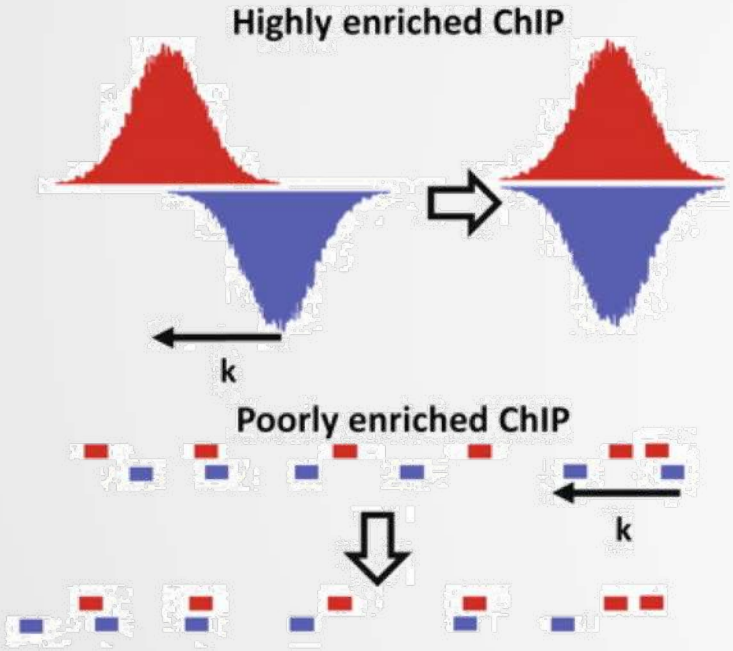
From reads to peaks

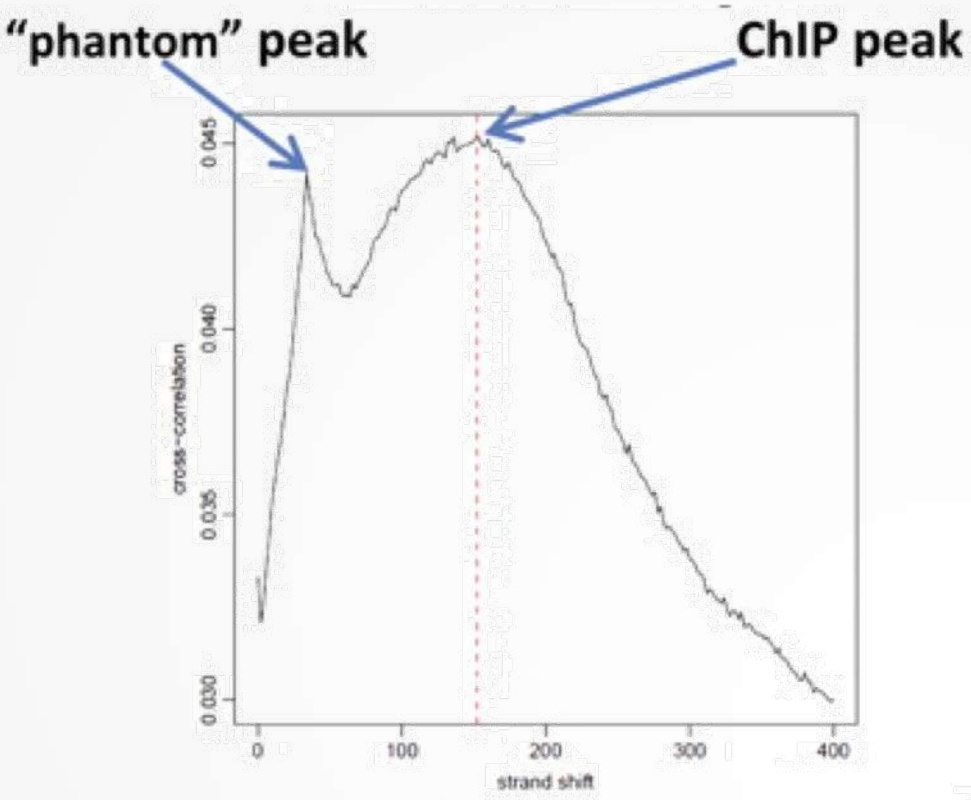
- Get the signal at the right position
  - Read shift
  - Extension
- Estimate the fragment size
- Do paired-end



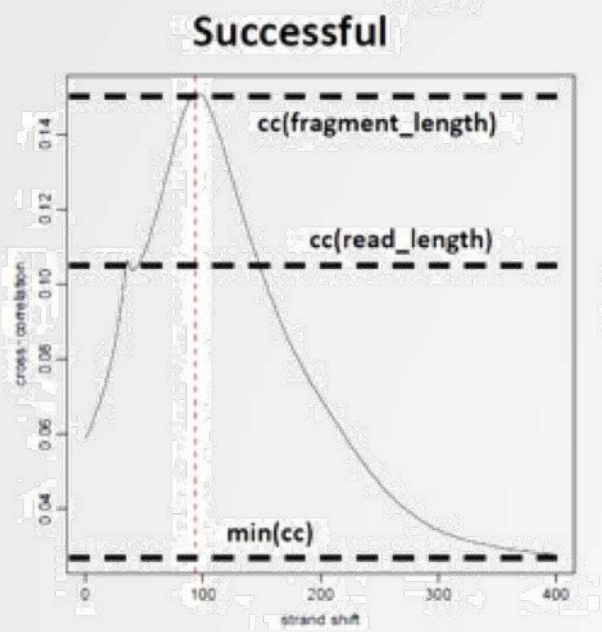
QC: cross correlation analysis

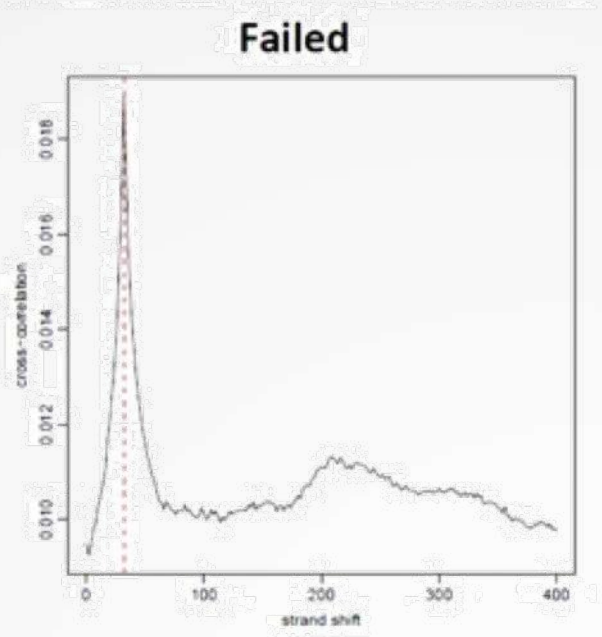
- The cross-correlation metric is computed as the Pearson's linear correlation between the Crick strand and the Watson strand, after shifting Watson by *k* base pairs.





QC: cross correlation analysis





NSC: normalized strand coefficient

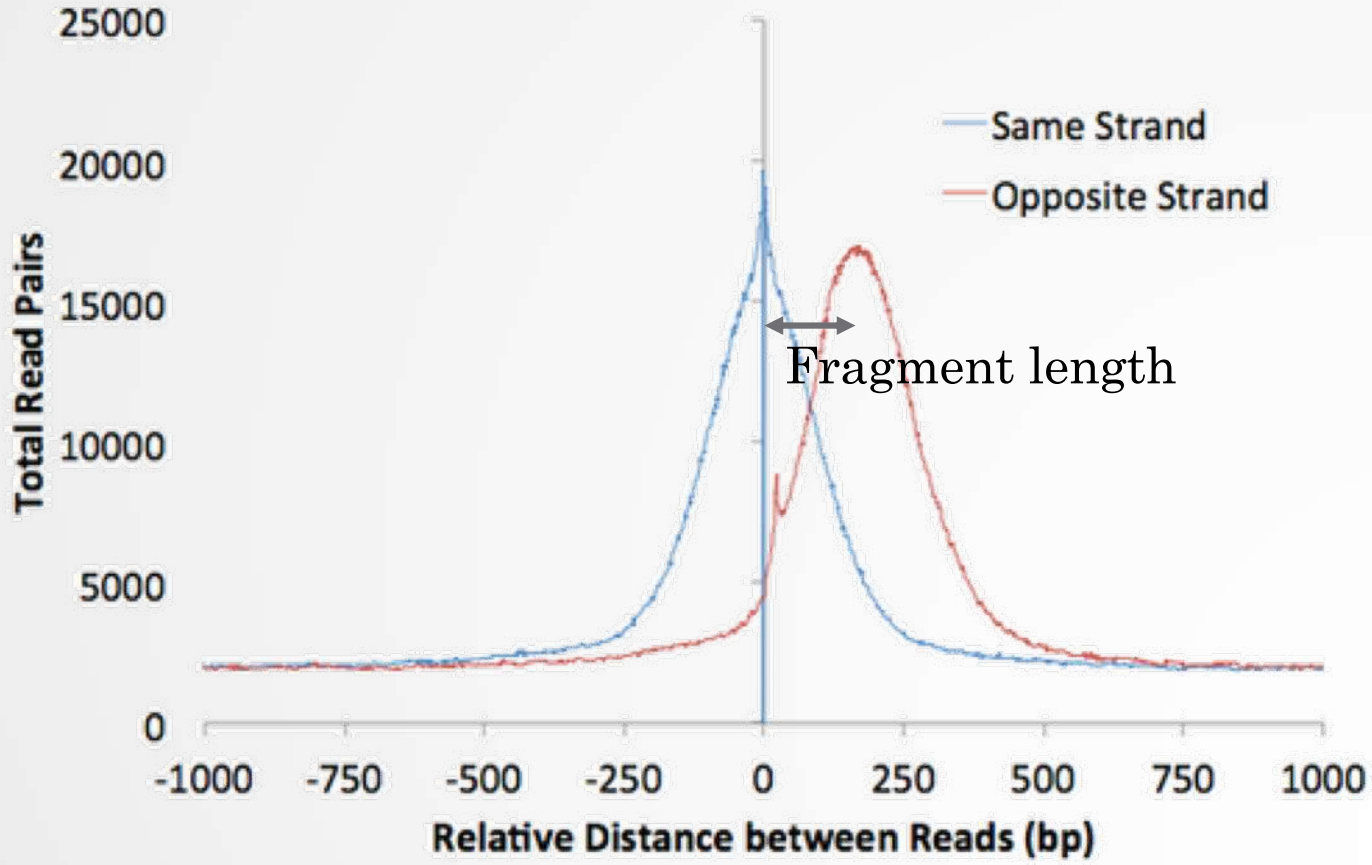
$$NSC = \frac{cc(fragment\ length)}{min(cc)}$$

Relative strand correlation (RSC)

$$RSC = \frac{cc(fragment\ length) - min(cc)}{cc(read\ length) - min(cc)}$$

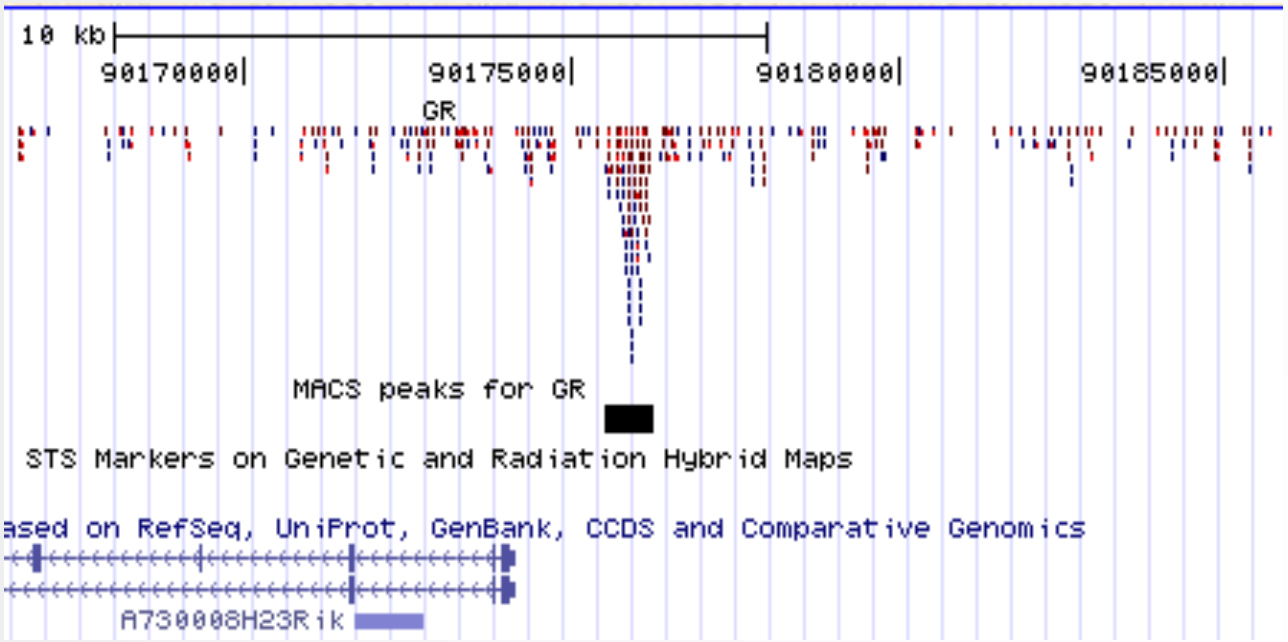
Estimating the fragment size

- Homer (Heinz et al, 2010): Compute distribution of distances between adjacent reads in the genome



Peak detection

- Discover interaction sites from aligned reads
- Idea: loci with a lot of reads/fragments = signal site



Peak detection

- Loci with lots of reads could also be due to
  - Sequencing biases
  - Chromatin biases (e.g CNVs)
  - PCR biases/artefacts
  - Biases/artefacts of unknown origin
  - So need to separate signal from noise
- Need to use a control to correct for the biases (Expect that the biaises are similar in input and in IP)

Peak finders

Pepke et al, 2009

	Profile	Peak criteria[a]	Tag shift	Control data[b]	Rank by	FDR[c]	User input parameters[d]	Artifact filtering: strand-based/ duplicate[e]	Refs.
CisGenome v1.1	Strand-specific window scan	1: Number of reads in window 2: Number of ChIP reads minus control reads in window	Average for highest ranking peak pairs	Conditional binomial used to estimate FDR	Number of reads under peak	1: Negative binomial 2: conditional binomial	Target FDR, optional window width, window interval	Yes / Yes	10
ERANGE v3.1	Tag aggregation	1: Height cutoff High quality peak estimate, per-region estimate, or input	High quality peak estimate, per-region estimate, or input	Used to calculate fold enrichment and optionally *P* values	*P* value	1: None 2: $\frac{\#\ \text{control}}{\#\ \text{ChIP}}$	Optional peak height, ratio to background	Yes / No	4,18
FindPeaks v3.1.9.2	Aggregation of overlapped tags	Height threshold	Input or estimated	NA	Number of reads under peak	1: Monte Carlo simulation 2: NA	Minimum peak height, subpeak valley depth	Yes / Yes	19
F-Seq v1.82	Kernel density estimation (KDE)	*s* s.d. above KDE for 1: random background, 2: control	Input or estimated	KDE for local background	Peak height	1: None 2: None	Threshold s.d. value, KDE bandwidth	No / No	14
GLITR	Aggregation of overlapped tags	Classification by height and relative enrichment	User input tag extension	Multiply sampled to estimate background class values	Peak height and fold enrichment	2: $\frac{\#\ \text{control}}{\#\ \text{ChIP}}$	Target FDR, number nearest neighbors for clustering	No / No	17
MACS v1.3.5	Tags shifted then window scan	Local region Poisson *P* value	Estimate from high quality peak pairs	Used for Poisson fit when available	*P* value	1: None 2: $\frac{\#\ \text{control}}{\#\ \text{ChIP}}$	*P*-value threshold, tag length, mfold for shift estimate	No / Yes	13
PeakSeq	Extended tag aggregation	Local region binomial *P* value	Input tag extension length	Used for significance of sample enrichment with binomial distribution	*q* value	1: Poisson background assumption 2: From binomial for sample plus control	Target FDR	No / No	5
QuEST v2.3	Kernel density estimation	2: Height threshold, background ratio	Mode of local shifts that maximize strand cross-correlation	KDE for enrichment and empirical FDR estimation	*q* value	1: NA 2: $\frac{\#\ \text{control}}{\#\ \text{ChIP}}$ as a function of profile threshold	KDE bandwidth, peak height, subpeak valley depth, ratio to background	Yes / Yes	9
SICER v1.02	Window scan with gaps allowed	*P* value from random background model, enrichment relative to control	Input	Linearly rescaled for candidate peak rejection and *P* values	*q* value	1: None 2: From Poisson *P* values	Window length, gap size, FDR (with control) or *E*-value (no control)	No / Yes	15
SiSSRs v1.4	Window scan	$N_+ - N_-$ sign change, $N_+ +$	Average nearest paired tag distance	Used to compute fold-enrichment distribution	*P* value	1: Poisson 2: control	1: FDR 1,2: $N_+ + N_-$	Yes / Yes	11

Peak finders

Basic components of peak callers:

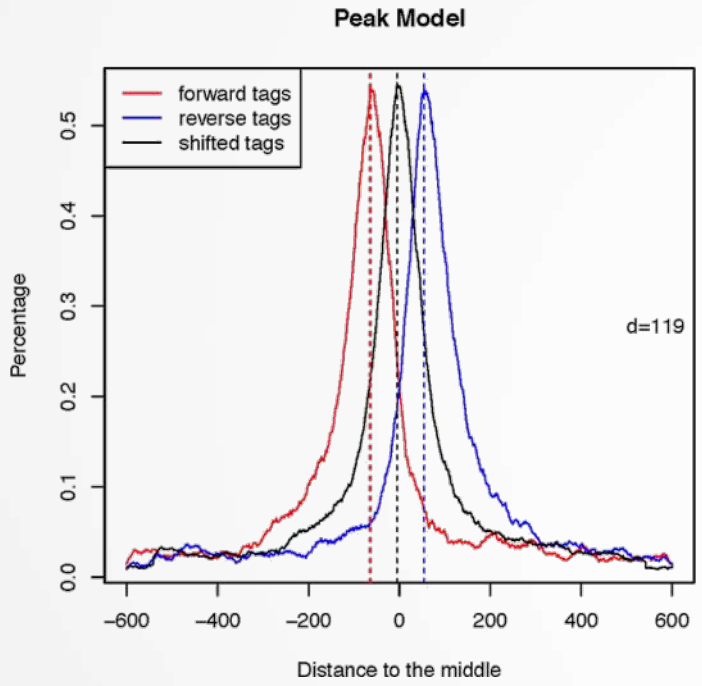
- A signal profile definition along each chromosome
- A background model
- Peak call criteria
- Post-call filtering of artifactual peaks
- Significance ranking of called peaks

Pepke et al, 2009

MACS [Zhang et al, 2008]

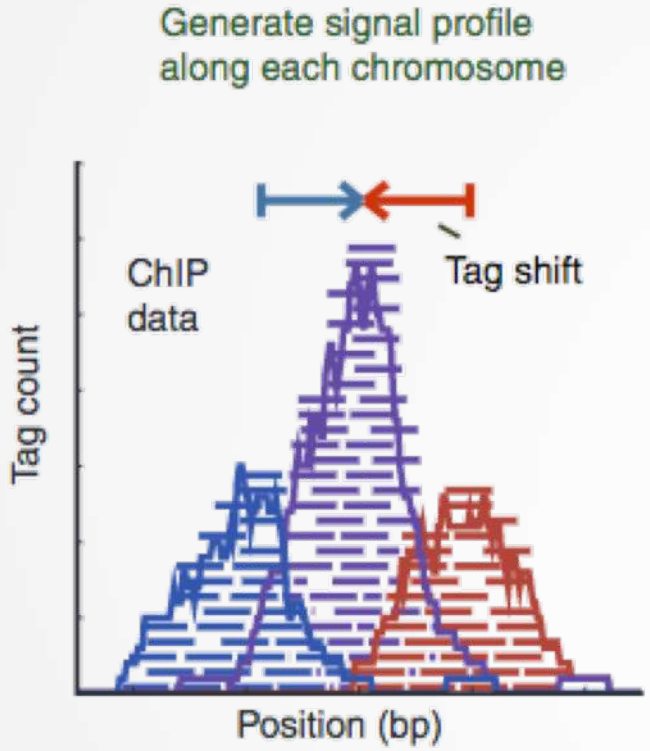
1. Modeling the shift size of ChIP-Seq tags

- slides *2bandwidth* windows across the genome to find regions with tags more than *mfold* enriched relative to a random tag genome distribution
- randomly samples 1,000 of these highly enriched peaks
- separates their Watson and Crick tags, and aligns them by the midpoint between their Watson and Crick tag centers
- define *d* as the distance in bp between the summit of the two distributions



MACS [Zhang et al, 2008]

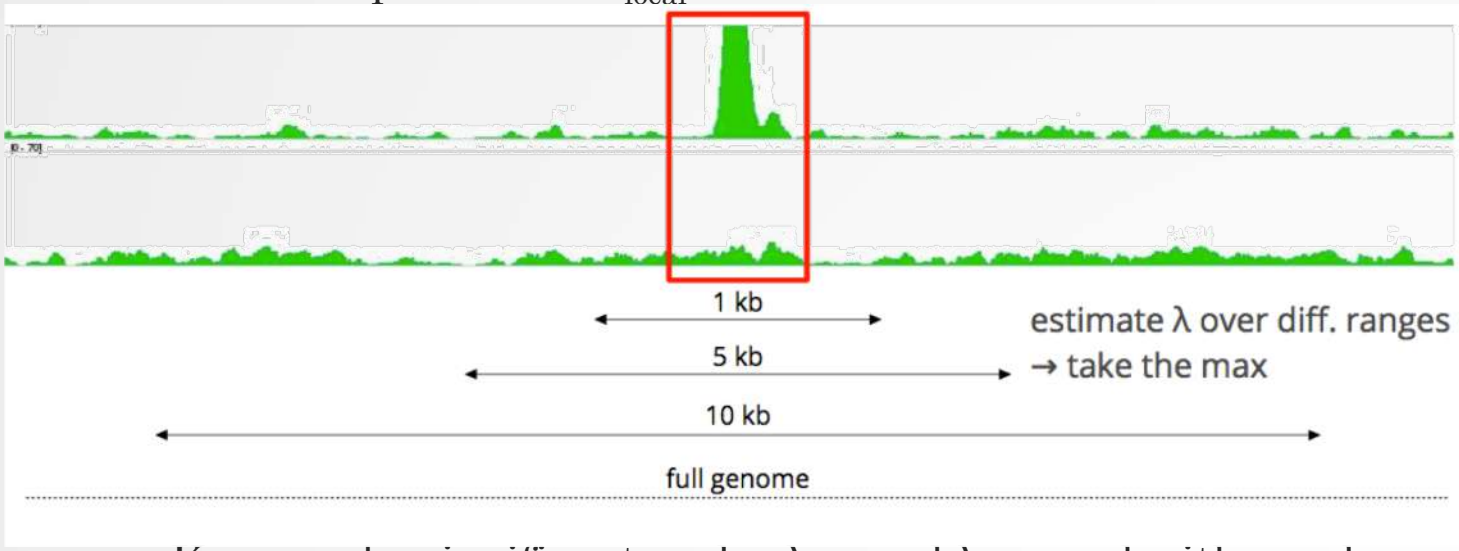
- **2. Peak detection**
  - Normalization: linearly scales the total control read count to be the same as the total ChIP read count
  - Duplicate read removal
  - Tags are shifted by *d*/2



Pepke et al, 2009

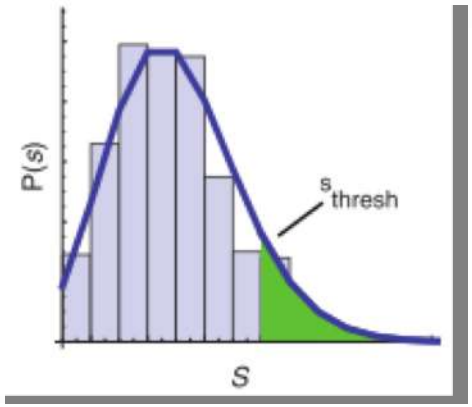
MACS [Zhang et al, 2008]

- Slides 2d windows across the genome to find candidate peaks with a significant tag enrichment (Poisson distribution *p*-value based on λ_{BG}, default 10^{-5})
- Estimate parameter λ_{local} of Poisson distribution



Source:
C. Herrmann

- Keep peaks significant under λ_{BG} and λ_{local} and with p-value < threshold



MACS [Zhang et al, 2008]

3. **Multiple testing correction (FDR)**

- Swap treatment and input and call negative peaks
- Take all the peaks (neg + pos) and sort them by increasing p-values

$$FDR(p) = \frac{\#\ \textbf{Negative}\ \text{peaks with p-value} < p}{\#\ \textbf{Selected peaks}}$$

FDR = 2/27 = 0.074

Exercise: peak calling

We now want to call MITF peaks.

- 1. Use **Macs2 callpeak** to perform the peak calling on the data. Use default parameters except for
  - ChIP-Seq Treatment File: mitf.bam
  - ChIP-Seq Control File: ctrl.bam
  - Effective genome size: Human
  - Outputs: Peaks as tabular file, summits, Summary page (html), Plot in PDF

Exercise: peak calling

- 2. Macs2 callpeak generates 5 datasets:
  - List of the peaks (tabular format)

List of arguments used to run Macs2

Peaks

	A	B	C	D	E	F	G	H	I	J
1	# This file is generated by MACS version 2.1.0.20151222									
2	# Command line: callpeak --name MACS2 -t /galaxy13/files/052/dataset_52866.dat -c /galaxy22/files/052/dataset_52865.dat --fo									
3	# ARGUMENTS LIST:									
4	# name = MACS2									
5	# format = BAM									
6	# ChIP-seq file = ['/galaxy13/files/052/dataset_52866.dat']									
7	# control file = ['/galaxy22/files/052/dataset_52865.dat']									
8	# effective genome size = 2.45e+09									
9	# band width = 300									
10	# model fold = [5, 50]									
11	# qvalue cutoff = 5.00e-02									
12	# Larger dataset will be scaled towards smaller dataset.									
13	# Range for calculating regional lambda is: 1000 bps and 10000 bps									
14	# Broad region calling is off									
15	# tag size is determined as 54 bps									
16	# total tags in treatment: 23124393									
17	# tags after filtering in treatment: 6223075									
18	# maximum duplicate tags at the same position in treatment = 1									
19	# Redundant rate in treatment: 0.73									
20	# total tags in control: 19949607									
21	# tags after filtering in control: 4798380									
22	# maximum duplicate tags at the same position in control = 1									
23	# Redundant rate in control: 0.76									
24	# d = 75									
25	# alternative fragment length(s) may be 75 bps									
26	chr	start	end	length	abs_summit	pileup	-log10(pvalue)	fold_enrichment	-log10(qvalue)	name
27	chr1	980686	980816	131	980745	8.48	10.38277	7.29361	6.46786	MACS2_peak_1
28	chr1	983821	983925	105	983877	6.94	9.11038	6.77148	5.34984	MACS2_peak_2
29	chr1	1031344	1031475	132	1031406	6.17	6.82634	5.21345	3.25879	MACS2_peak_3
30	chr1	1079424	1079564	141	1079490	12.34	18.30659	10.88735	13.88358	MACS2_peak_4
31	chr1	1304817	1304958	142	1304891	13.11	20.10101	11.51679	15.56374	MACS2_peak_5

Exercise: peak calling

- 2. Macs2 callpeak generates 5 datasets:
  - List of the peaks (tabular format)

26	chr	start	end	length	abs_summit	pileup	-log10(pvalue)	fold_enrichment	-log10(qvalue)	name
27	chr1	980686	980816	131	980745	8.48	10.38277	7.29361	6.46786	MACS2_peak_1
28	chr1	983821	983925	105	983877	6.94	9.11038	6.77148	5.34984	MACS2_peak_2
29	chr1	1031344	1031475	132	1031406	6.17	6.82634	5.21345	3.25879	MACS2_peak_3
30	chr1	1079424	1079564	141	1079490	12.34	18.30659	10.88735	13.88358	MACS2_peak_4
31	chr1	1304817	1304958	142	1304891	13.11	20.10101	11.51679	15.56374	MACS2_peak_5

  - chr: chromosome name
  - start: start position of peak
  - end: end position of peak
  - length: length of peak region
  - abs_summit: absolute peak summit position
  - pileup: pileup height at peak summit
  - -log10(pvalue): -log10(pvalue) for the peak summit (e.g. pvalue =1e-10, then this value should be 10)
  - fold_enrichment: fold enrichment for this peak summit against random Poisson distribution with local lambda
  - -log10(qvalue): -log10(qvalue) at peak summit
  - name: peak name

Exercise: peak calling

- List of the peaks (Narrowpeak format)

1	2	3	4	5	6	7	8	9	10
chr1	980685	980816	MACS2_peak_1	64	.	7.29361	10.38277	6.46786	59
chr1	983820	983925	MACS2_peak_2	53	.	6.77148	9.11038	5.34984	56
chr1	1031343	1031475	MACS2_peak_3	32	.	5.21345	6.82634	3.25879	62
chr1	1079423	1079564	MACS2_peak_4	138	.	10.88735	18.30659	13.88358	66
chr1	1304816	1304958	MACS2_peak_5	155	.	11.51679	20.10101	15.56374	74
chr1	1441082	1441181	MACS2_peak_6	124	.	10.25923	16.71260	12.40068	71

1. chr
2. Start of peak
3. End of peak
4. Peak name
5. Integer score for display
7. fold-change
8. -log10pvalue
9. -log10qvalue
10. Relative summit position to peak start

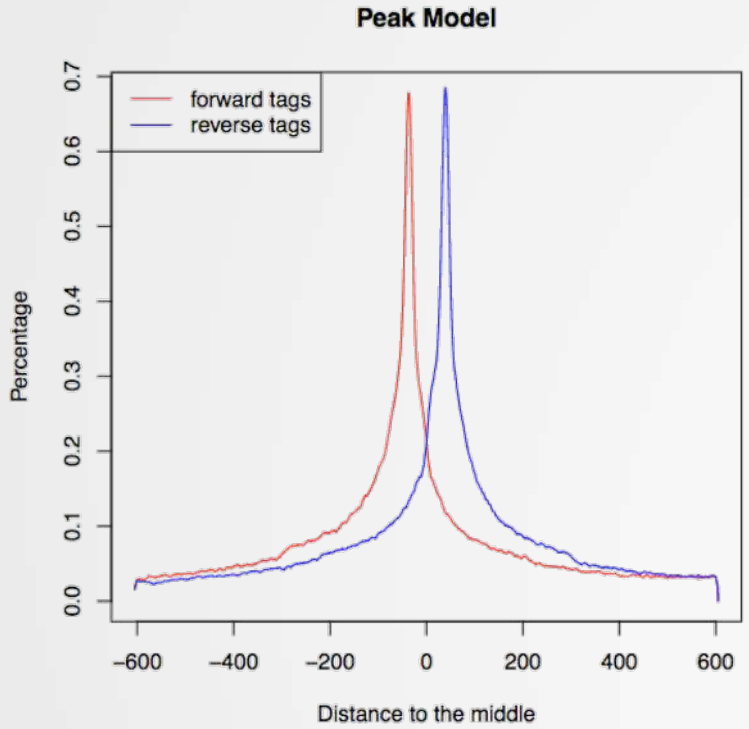
Exercise: peak calling

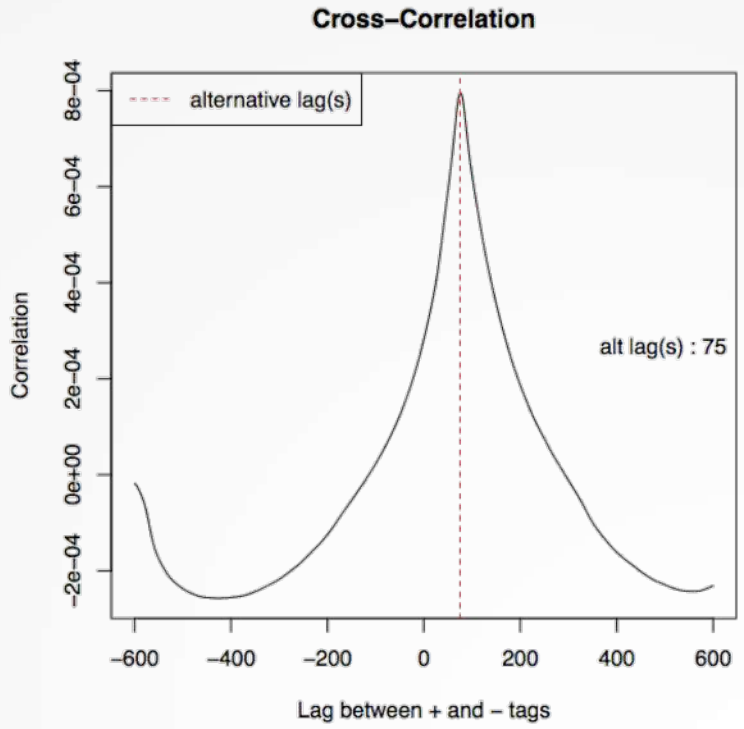
- List of the peak summits (BED): contains the peak summit location for each peak.

1. chr	2. Start of peak	3. End of peak	4. Peak name	5. -log10pvalue
1	2	3	4	5
chr1	980744	980745	MACS2_peak_1	6.46786
chr1	983876	983877	MACS2_peak_2	5.34984
chr1	1031405	1031406	MACS2_peak_3	3.25879
chr1	1079489	1079490	MACS2_peak_4	13.88358
chr1	1304890	1304891	MACS2_peak_5	15.56374
chr1	1441153	1441154	MACS2_peak_6	12.40068

Exercise: peak calling

- PDF images about the model based on your data





- Log of MACS - output during Macs2 run (HTML)

Exercise: peak calling

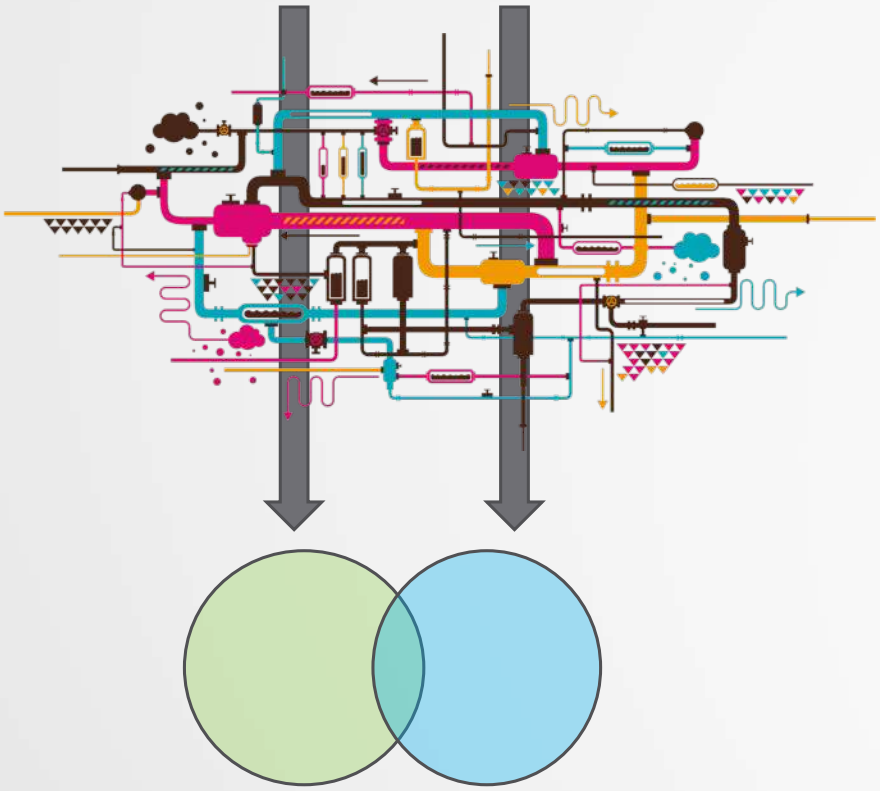
We now want to call MITF peaks.

- 1. Use **Macs2 callpeak** to perform the peak calling on the data. Use default parameters except for
  - ChIP-Seq Treatment File: mitf.bam
  - ChIP-Seq Control File: ctrl.bam
  - Effective genome size: Human
  - Outputs: Peaks as tabular file, summits, Summary page (html), Plot in PDF
- 2. Look at the resulting datasets. How many peaks are found?
- 3. What is the fragment size estimated by Macs2? What do you think of the value?
- 4. Rerun **Macs2** using the same parameters as before but changing the shift size:
  - Build Model: Do not build the shifting model (--nomodel)
  - The arbitrary extension size in bp: 100
- 5. How many peaks are now found?

How to deal with replicates

Analyze samples separately and takes union or intersection of resulting peaks

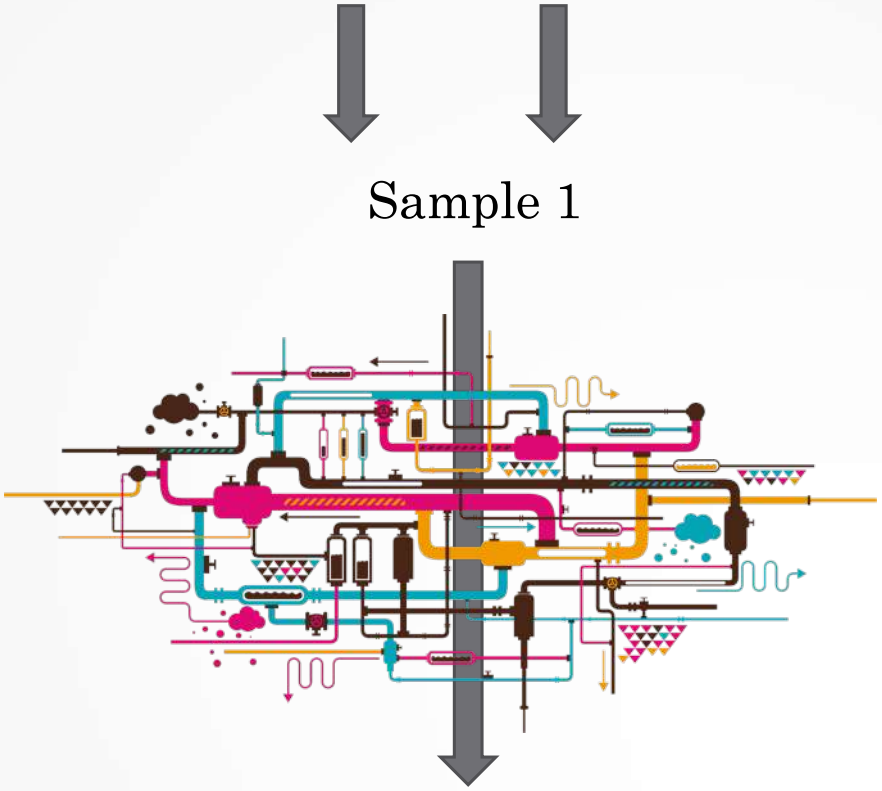
Sample 1.a Sample 1.b



Merge samples prior to the peak calling (e.g recommended by MACS)

Sample 1.a Sample 1.b

Sample 1

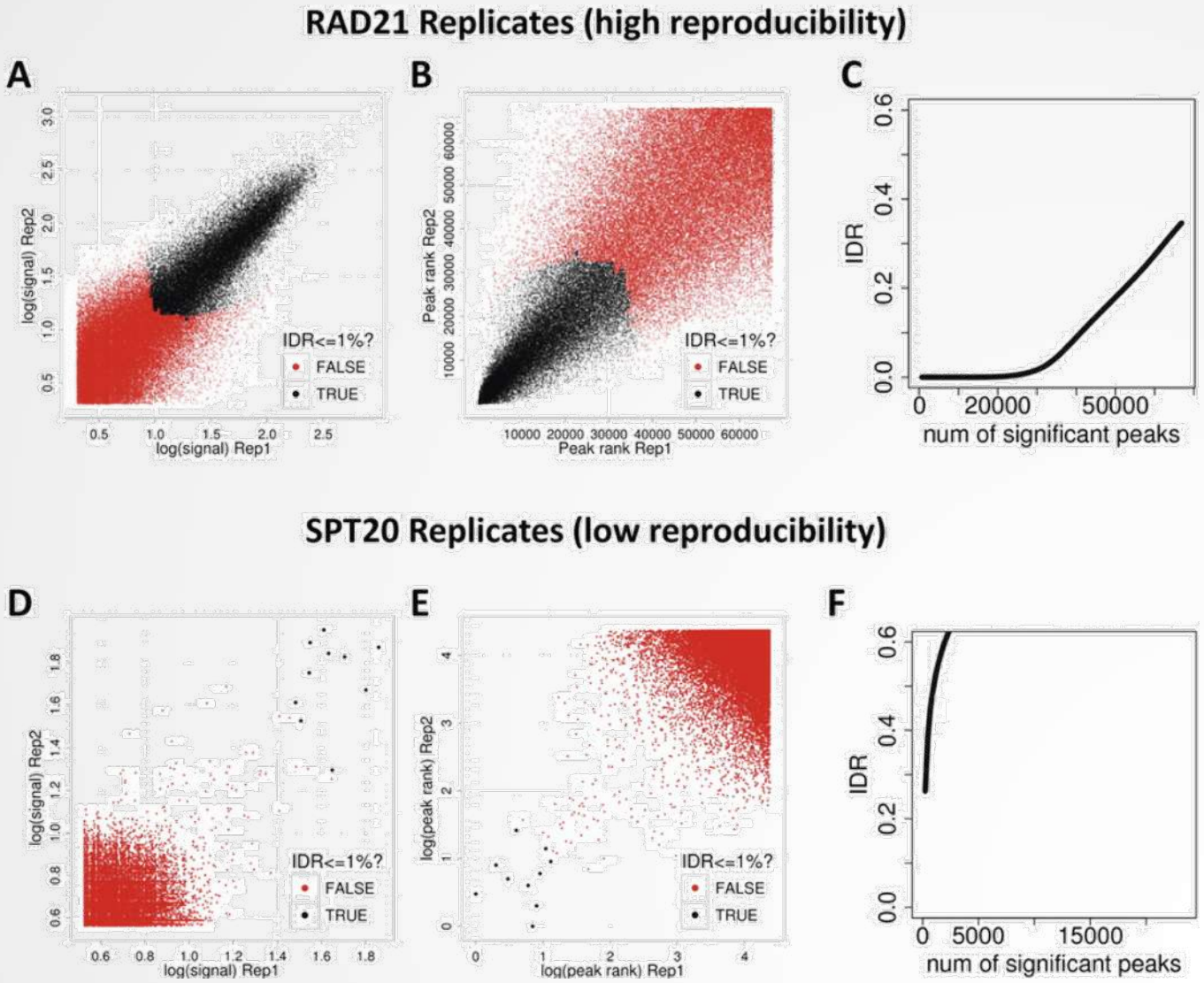


IDR

- Measures consistency between replicates
- Uses reproducibility in score rankings between peaks in each replicate to determine an optimal cutoff for significance.
- Idea:
  - The most significant peaks are expected to have high consistency between replicates
  - The peaks with low significance are expected to have low consistency

https://sites.google.com/site/anshulkundaje/projects/idr

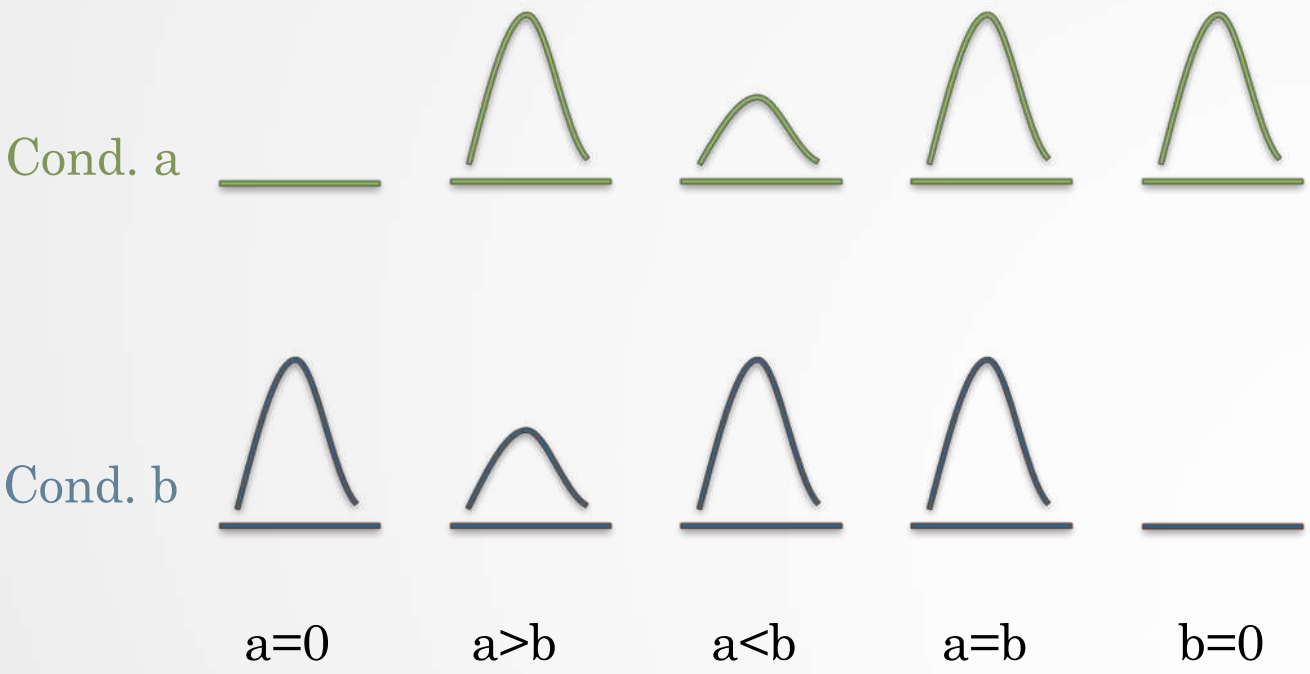
IDR



(!) IDR doesn't work on broad source data!

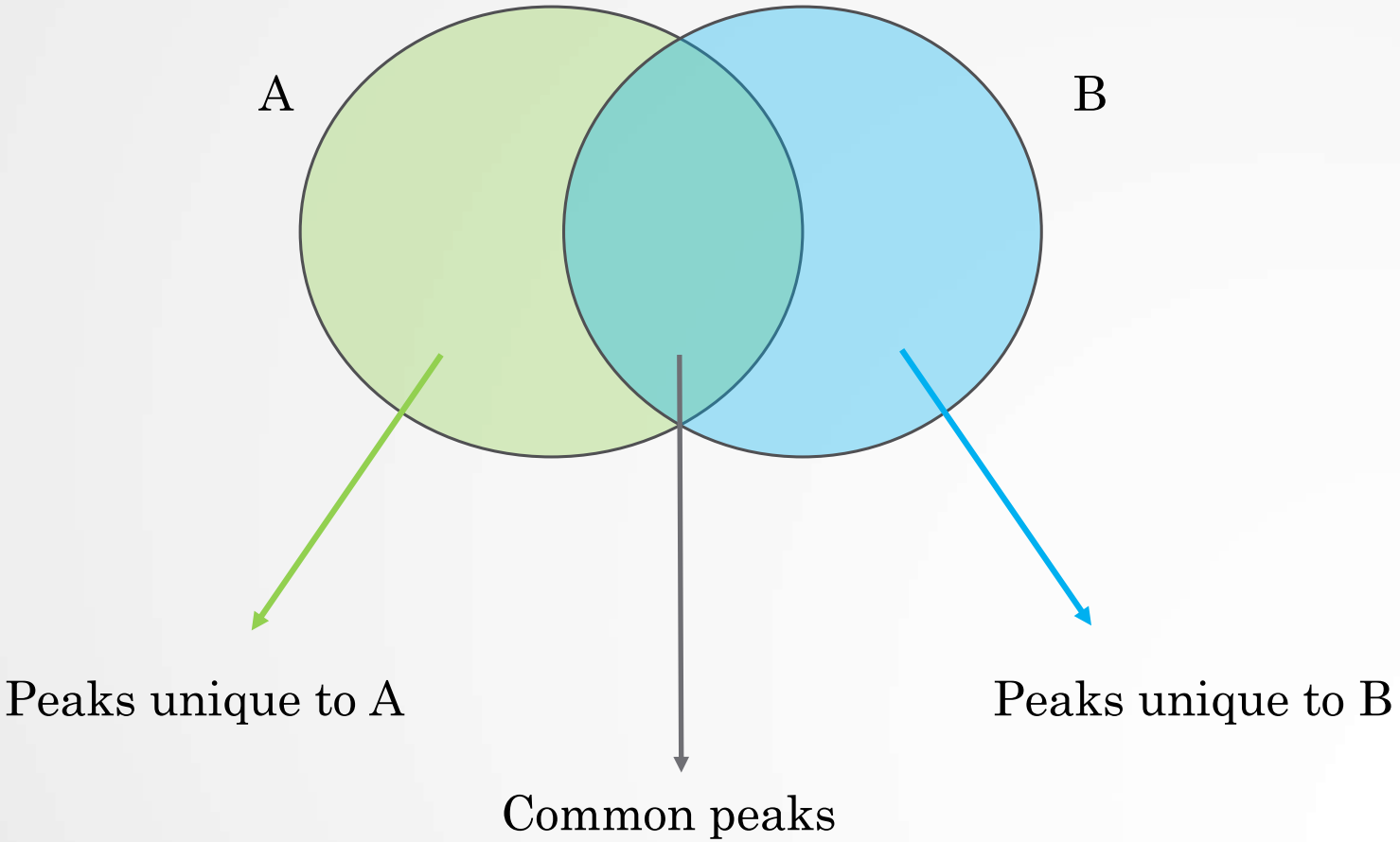
Differential binding analysis

- Find differential binding events by comparing different conditions
  - qualitative analysis: binding vs no binding
  - quantitative analysis: weak binding vs strong binding



Differential binding analysis

Qualitative approach



Differential binding analysis

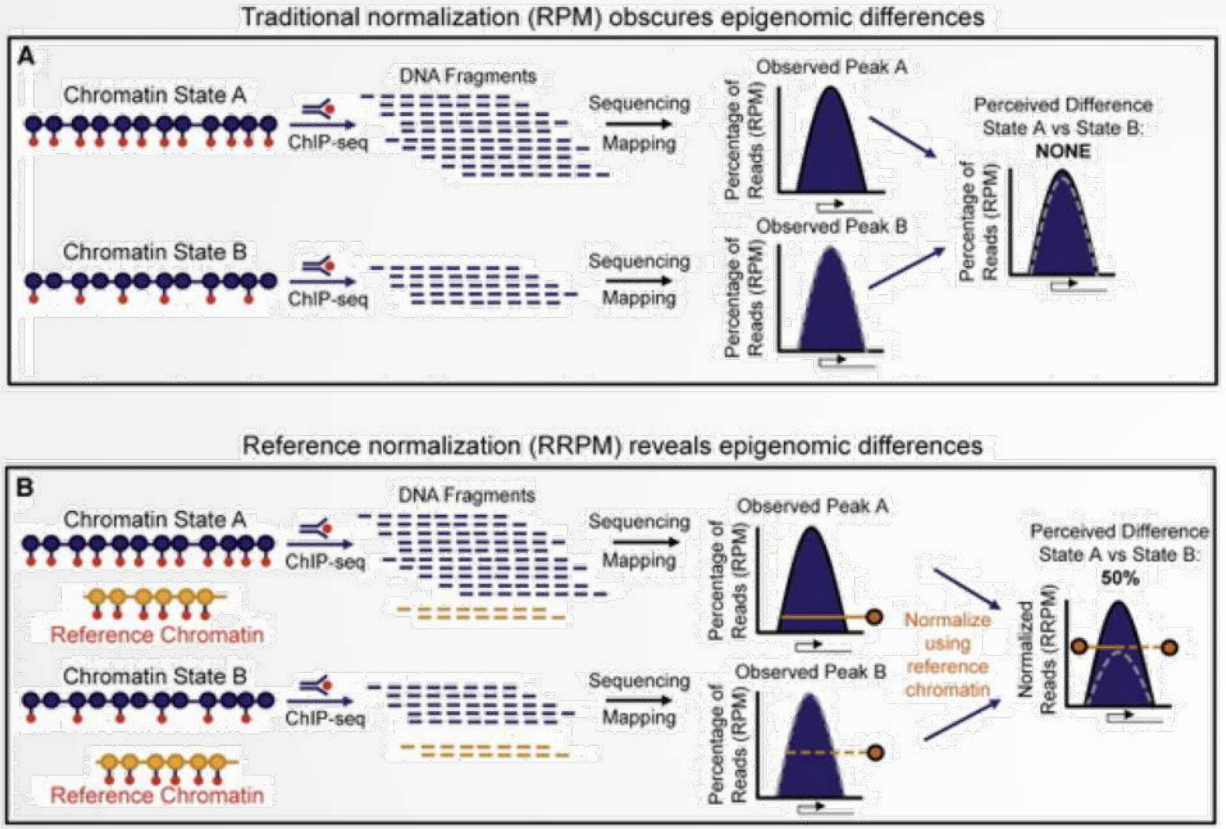
Quantitative approach

- Do the peak calling on all data
- Take union of all peaks
- Do quantitative analysis of differential binding events based on read counts

- Statistical models
  - No replicates: assume simple Poisson model
  - With replicates: perform differential test using DE tools from RNA-seq (EdgeR, DESeq,...) based on read counts

Spike-in

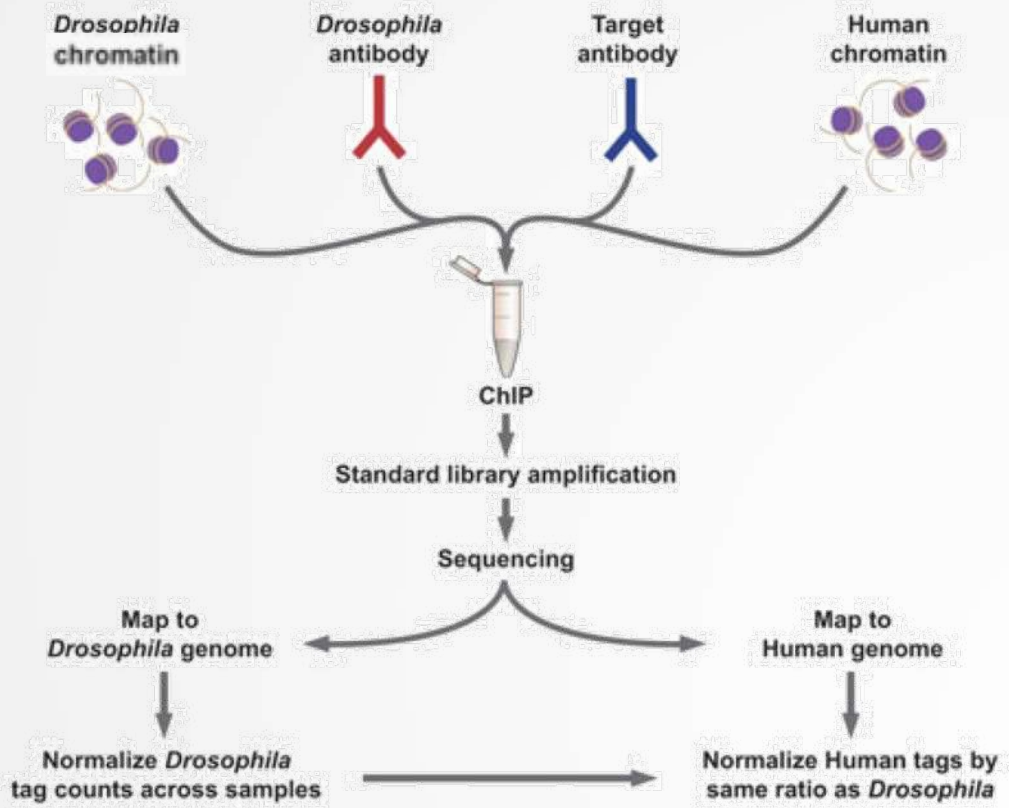
- Current normalization methods fail to detect global changes as they make the assumption that globally nothing change but a small portion of the genome
- Insert external chromatin used as reference chromatin



Orlando et al, 2014

Spike-in

- Spike-in normalization can be applied to ChIP-Seq data to reduce the effects of technical variation and sample processing bias



http://www.activemotif.com/catalog/1091/chip-normalization