Introduction to ChIP-seq data analysis

ENAR 2014

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Outline

- Introduction to ChIP-seq experiment: biological motivation and experimental procedure.
- Method and software for ChIP-seq peak calling:
  - Protein binding ChIP-seq.
  - Histone modifications.
- After peak calling:
  - Overlaps of peaks.
  - Differential analysis.

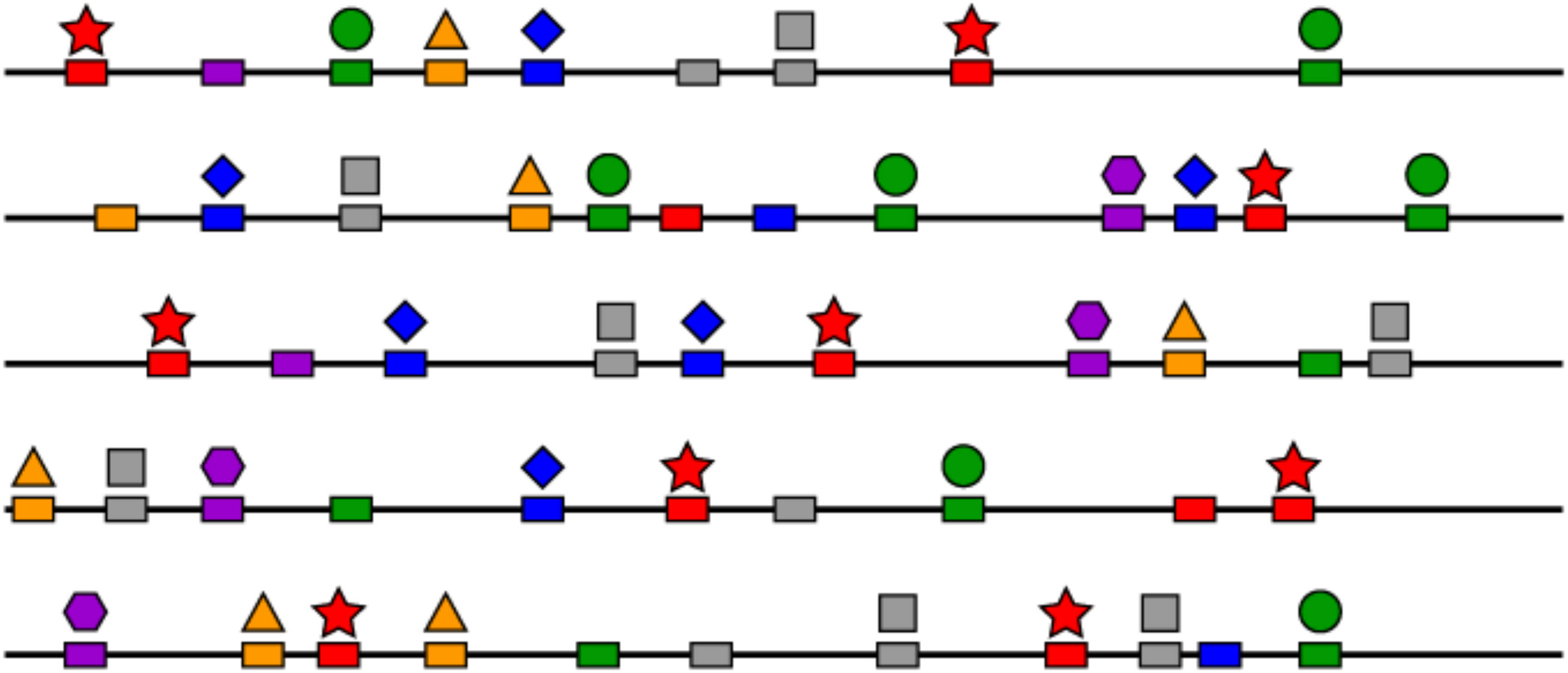
ChIP-seq: Chromatin ImmunoPrecipitation + sequencing

- Biological motivation: detect or measure some type of biological modifications along the genome:
  - Detect binding sites of DNA-binding proteins (transcription factors, pol2, etc.) .
  - Quantify strengths of chromatin modifications (e.g., histone modifications).

Experimental procedures

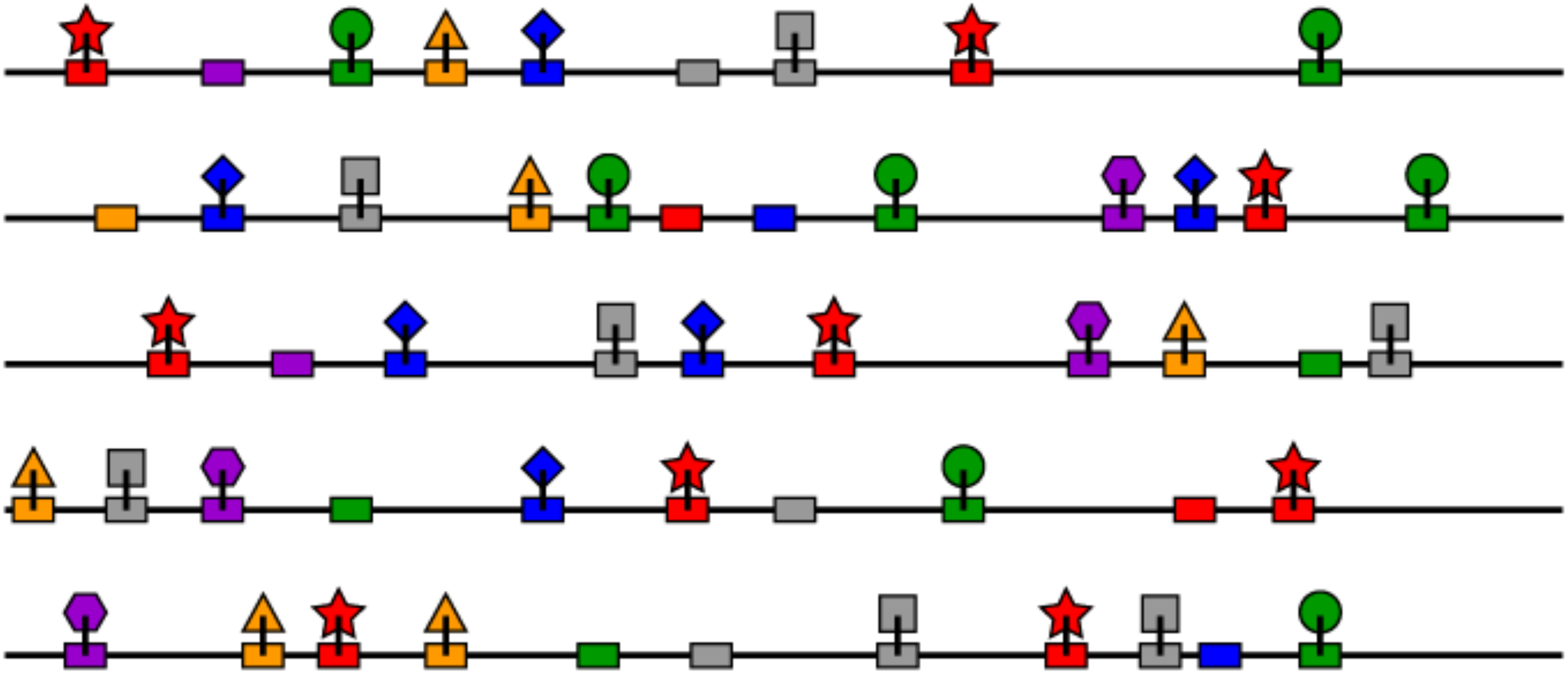
- **Crosslink**: fix proteins on isolate genomic DNA.
- **Sonication**: cut DNA in small pieces of ~200bp.
- **IP**: use antibody to capture DNA segments with specific proteins.
- **Reverse crosslink**: remove protein from DNA.
- Sequence the DNA segments.

Genomic DNA with TF



By Richard Bourgon at UC Berkley

TF/DNA Crosslinking *in vivo*



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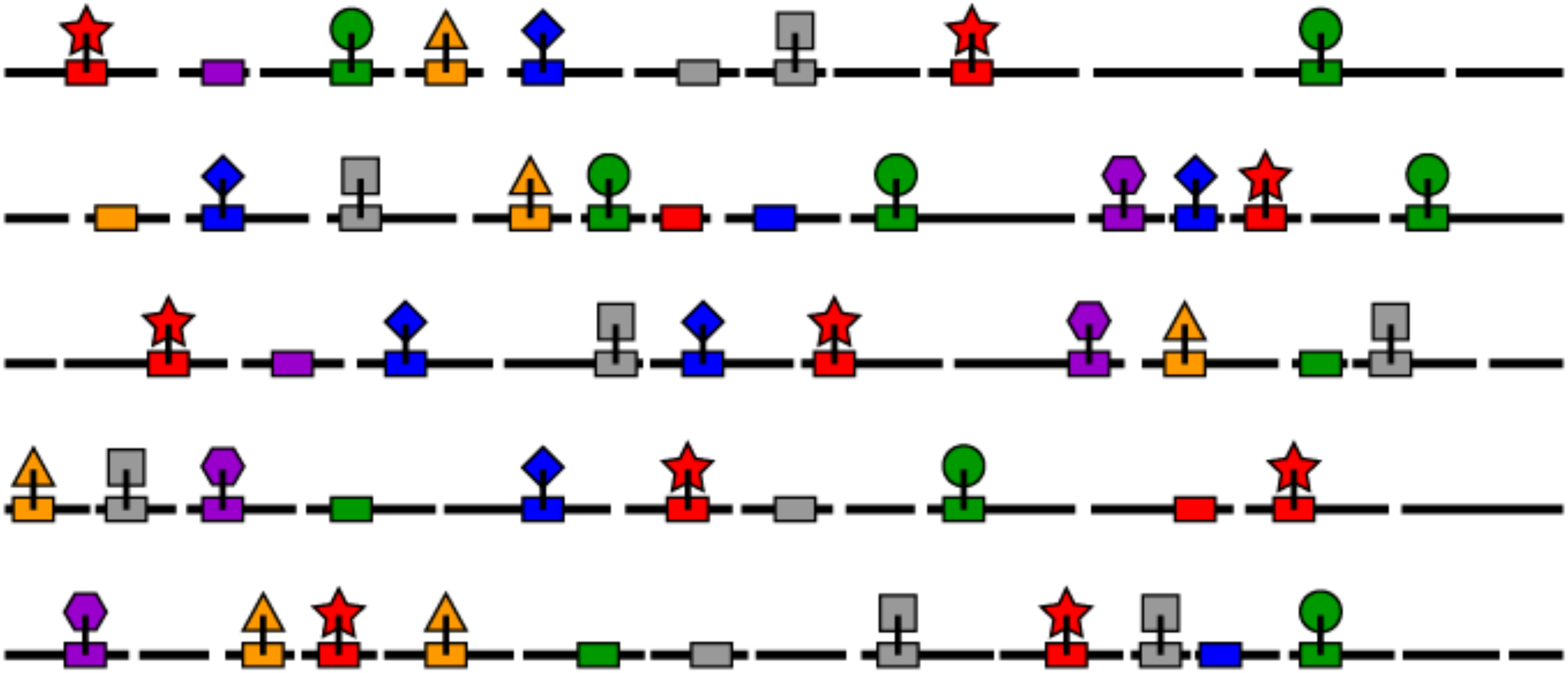
Sonication



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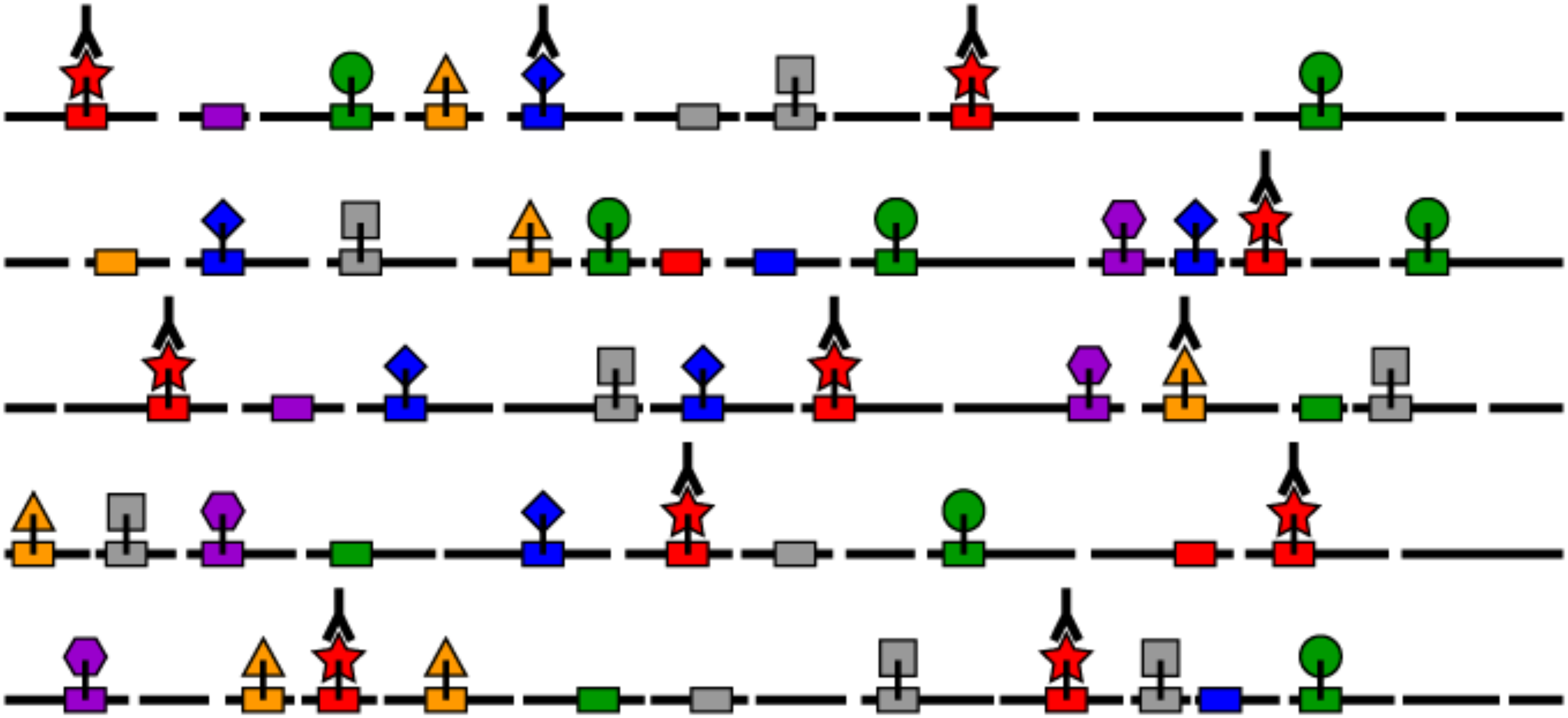
TF-specific Antibody



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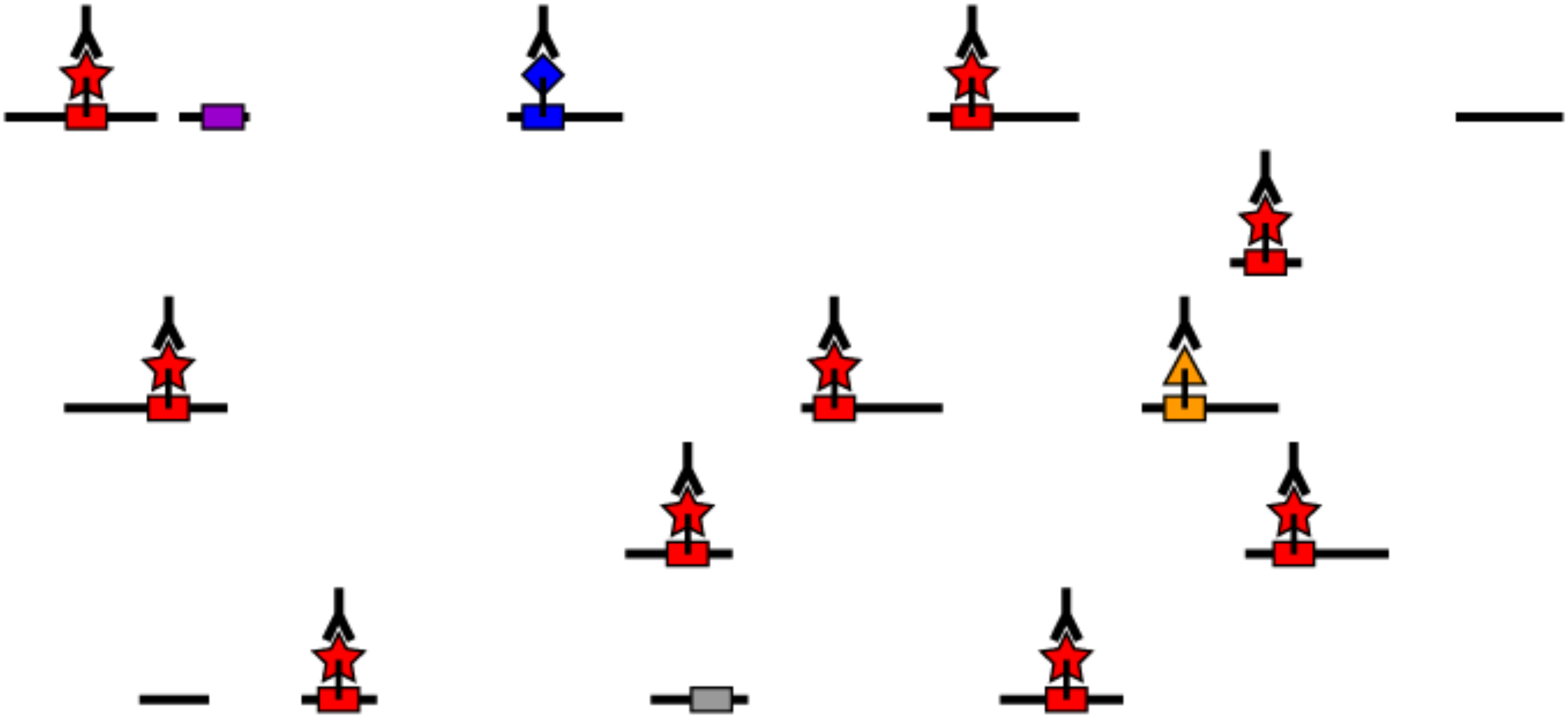
Immunoprecipitation (IP)



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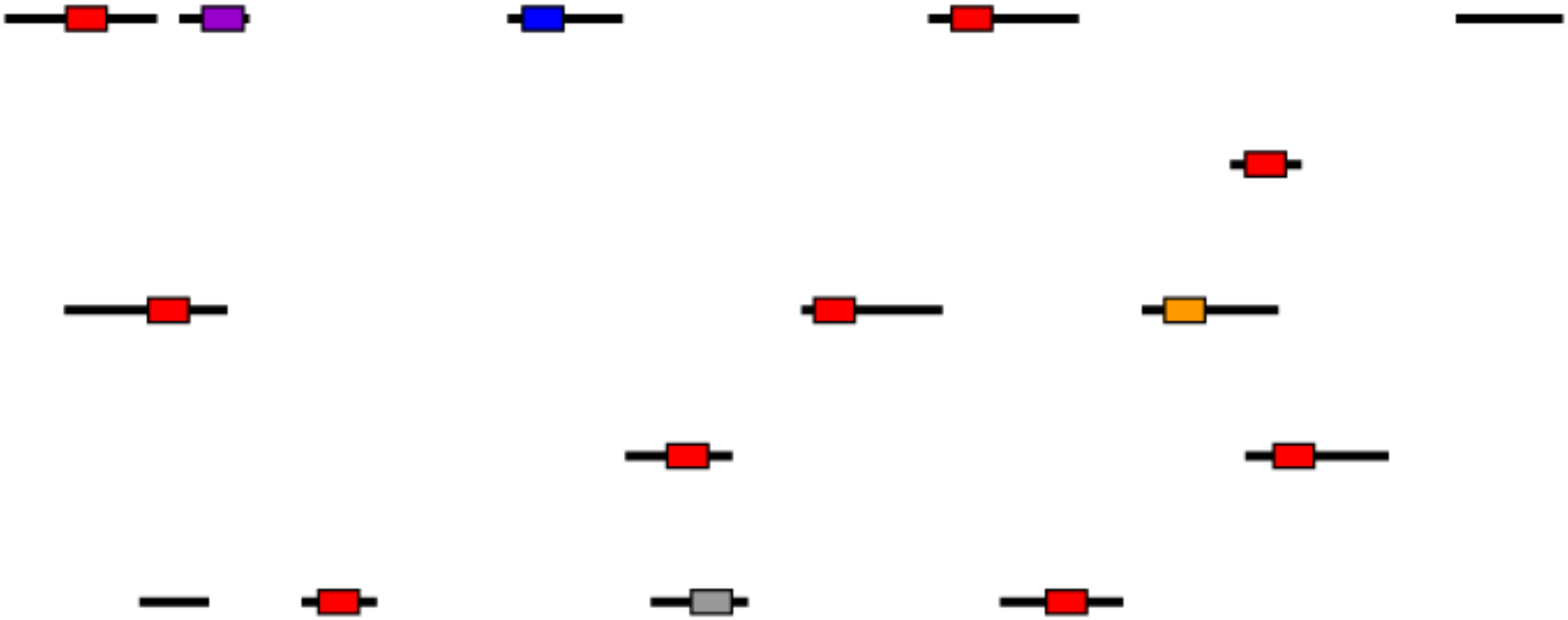
Reverse Crosslink and DNA Purification



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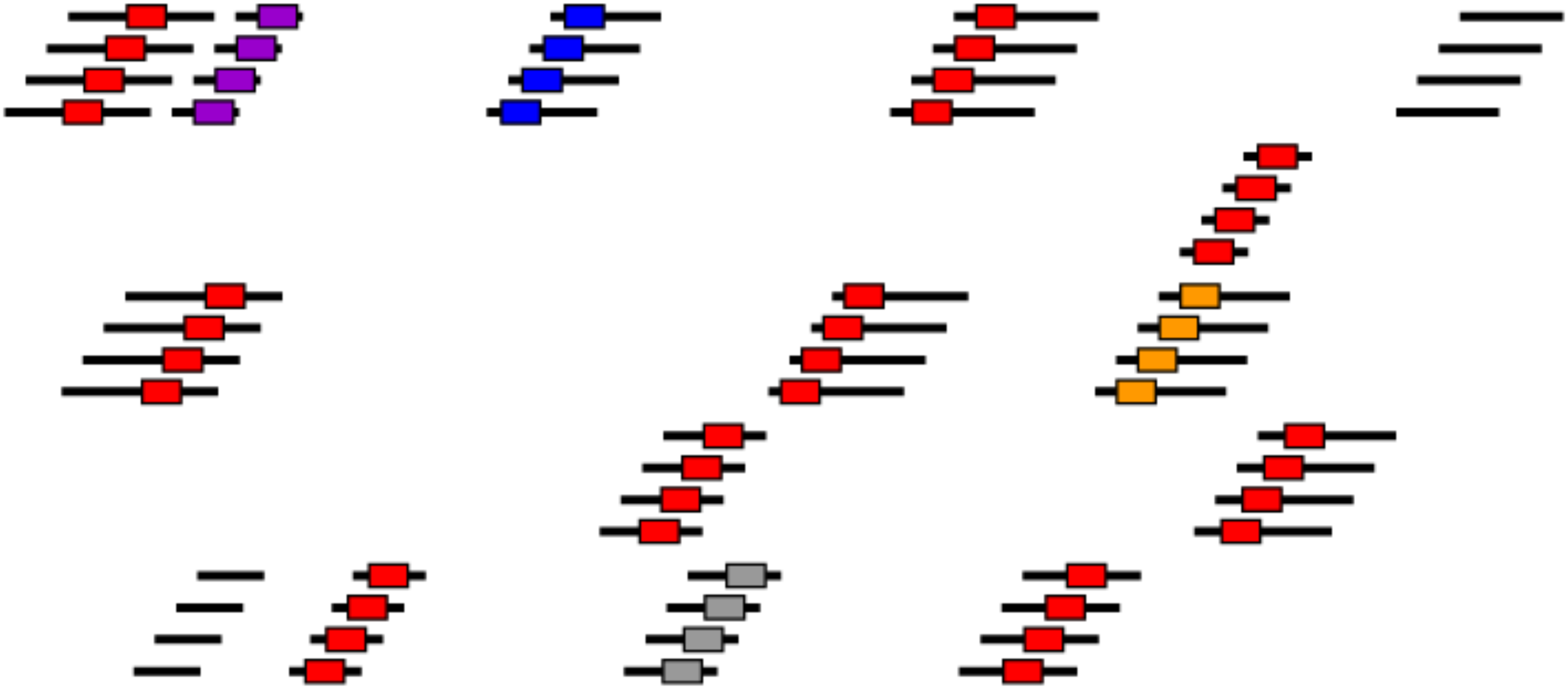
Amplification then sequencing



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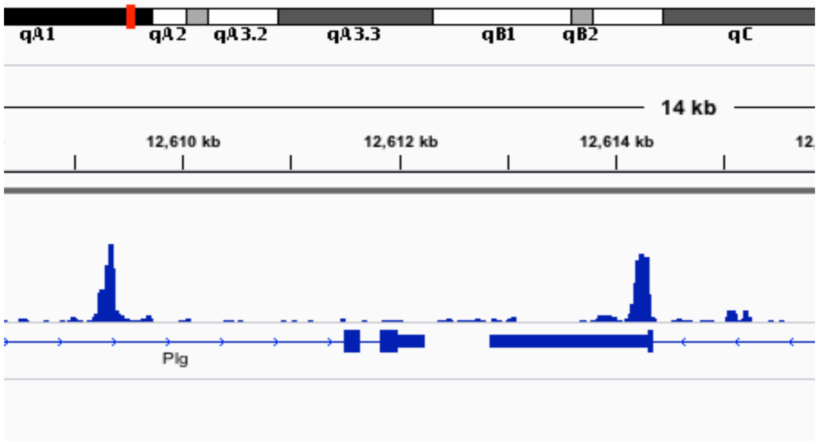
Data from ChIP-seq

- Raw data: sequence reads.
- After alignments: genome coordinates (chromosome/position) of all reads.
- For downstream analysis, aligned reads are often summarized into “counts” in equal sized bins genome-wide:
    1. segment genome into small bins of equal sizes (50bps).
    2. Count number of reads started at each bin.

Methods and software for ChIP-seq peak/block calling

ChIP-seq "peak" detection

- When plot the read counts against genome coordinates, the binding sites show a tall and pointy peak. So "peaks" are used to refer to protein binding or histone modification sites.



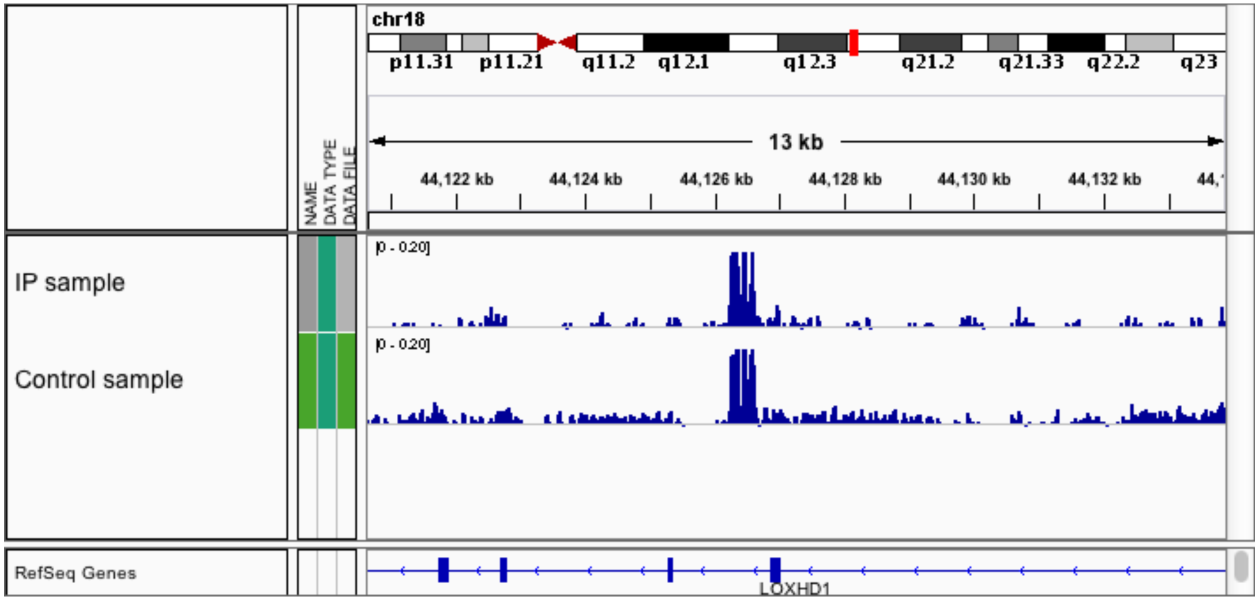
- Peak detection is the most fundamental problem in ChIP-seq data analysis.

Simple ideas for peak detection

- Peaks are regions with reads clustered, so they can be detected from binned read counts.
- Counts from neighboring windows need to be combined to make inference (so that it's more robust).
- To combine counts:
  - Smoothing based: moving average (MACS, CisGenome), HMM-based (Hpeak).
  - Model clustering of reads starting position (PICS, GPS).
- Moreover, some special characteristics of the data can be considered to improve the peak calling performance.

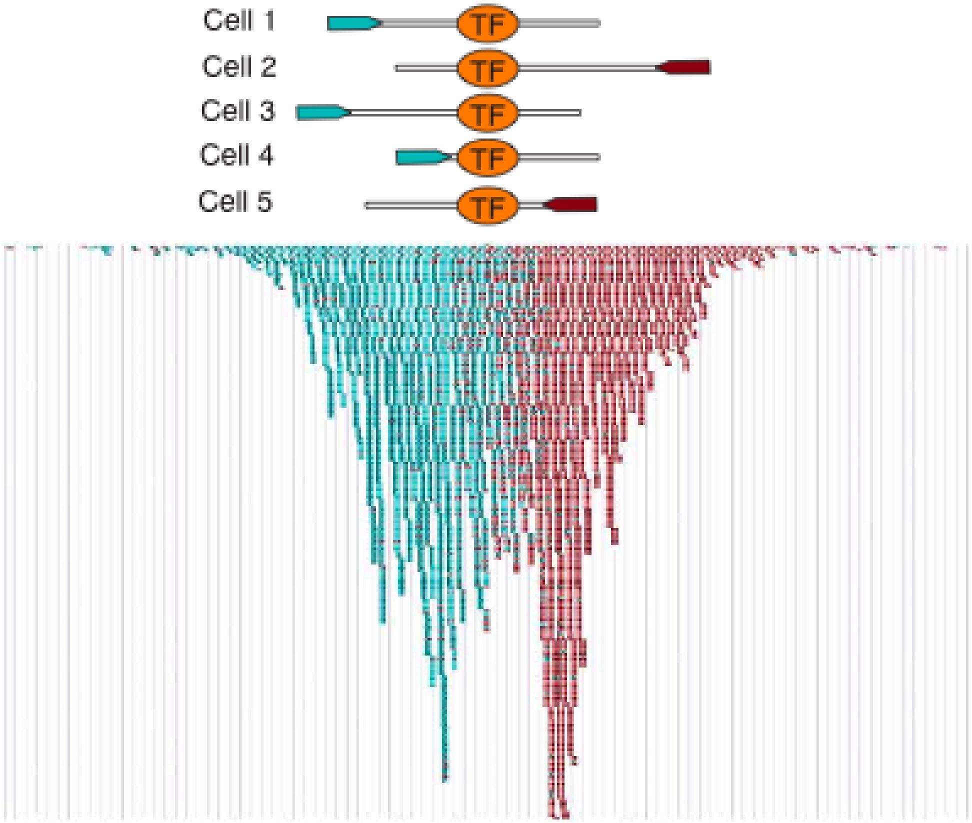
Control sample is important

- A control sample is necessary for correcting many artifacts:
  - DNA sequence contents affect amplification or sequencing process.
  - Repetitive regions affect alignments.
  - Chromatin structures (e.g., open chromatin region or not) affect the DNA sonication process.



Reads aligned to different strands

- Number of Reads aligned to different strands form two distinct peaks around the true binding sites.
- This information can be used to help peak detection.



Valouev et al. (2008) *Nature Method*

Mappability

- For each basepair position in the genome, whether a 35 bp sequence tag starting from this position can be uniquely mapped to a genome location.
- Regions with low mappability (highly repetitive) cannot have high counts (because multi-aligned reads are discarded), thus affect the ability to detect peaks.

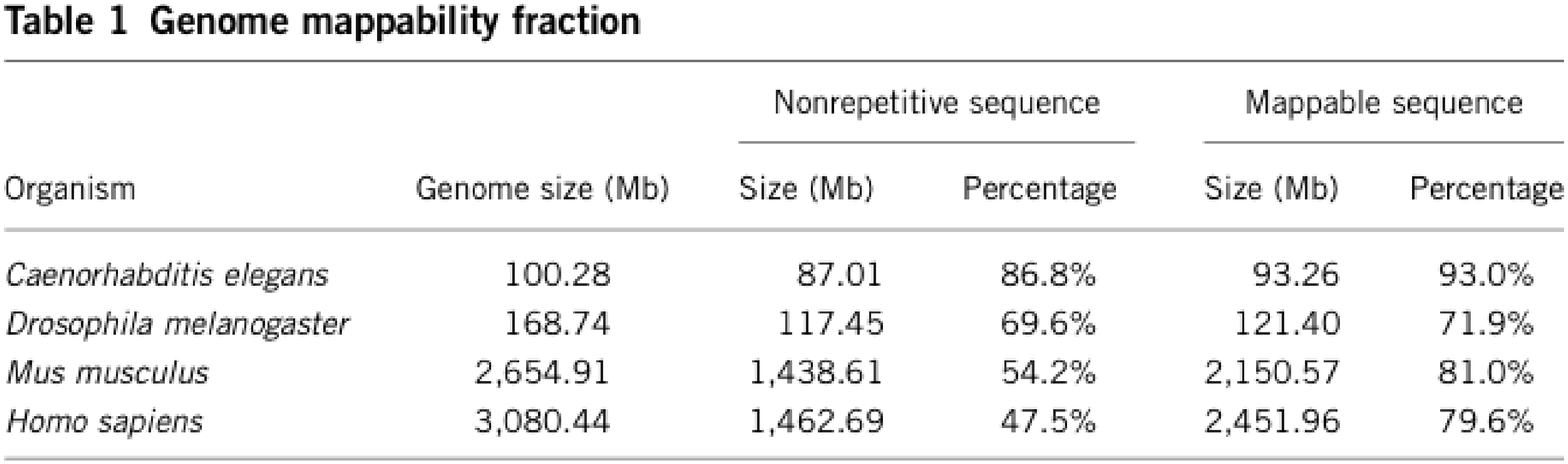


Table 1 Genome mappability fraction

		Nonrepetitive sequence		Mappable sequence	
Organism	Genome size (Mb)	Size (Mb)	Percentage	Size (Mb)	Percentage
Caenorhabditis elegans	100.28	87.01	86.8%	93.26	93.0%
Drosophila melanogaster	168.74	117.45	69.6%	121.40	71.9%
Mus musculus	2,654.91	1,438.61	54.2%	2,150.57	81.0%
Homo sapiens	3,080.44	1,462.69	47.5%	2,451.96	79.6%

Normalization issues

- The most common normalization needed is to adjust for total counts.
- Normalize by total counts is conservative, because ChIP sample contains reads mapped to background and peaks, but control sample have reads mapped to background only.
- It's better to normalize using the number of total reads in backgrounds. Two pass algorithm:
  - Roughly find peaks, and exclude those regions.
  - Compute total reads in the leftover regions and normalize based on that.
- Other normalizations (GC contents, MA plot based) available, but don't seems to help much.

Peak detection software

- MACS
- Cisgenome
- QuEST
- Hpeak
- PICS
- GPS
- PeakSeq
- MOSAiCS
- …

MACS (Model-based Analysis of ChIP-Seq) Zhang et al. 2008, *GB*

- Estimate shift size of reads *d* from the distance of two modes from + and – strands.
- Shift all reads toward 3' end by *d/2*.
- Use a dynamic Possion model to scan genome and score peaks. Counts in a window are assumed to following Poisson distribution with rate: $\lambda_{local} = \max(\lambda_{BG}, [\lambda_{1k},] \lambda_{5k}, \lambda_{10k})$
  - The dynamic rate capture the local fluctuation of counts.
- FDR is estimated from sample swapping: flip the IP and control samples and call peaks. Number of peaks detected under each p-value cutoff will be used as null and used to compute FDR.

Using MACS is easy

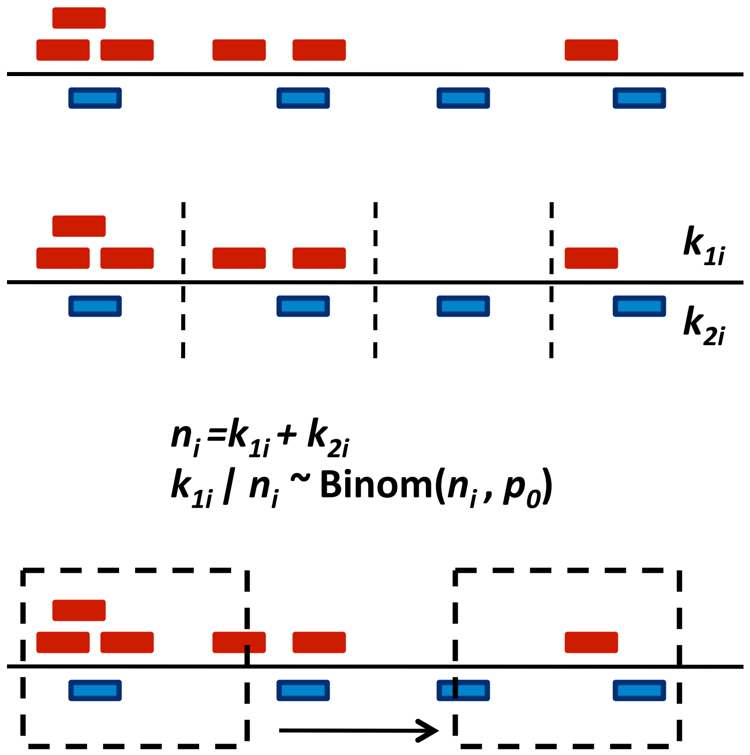
- http://liulab.dfci.harvard.edu/MACS/index.html
- Written in Python, runs in command line.
- Command:

```
macs14 -t sample.bed -c control.bed -n result
```

- A problem: doesn’t consider replicates. Data from replicated samples need to be merged.

Cisgenome (Ji et al. 2008, *NBT*)

- Implemented with Windows GUI.
- Use a Binomial model to score peaks.



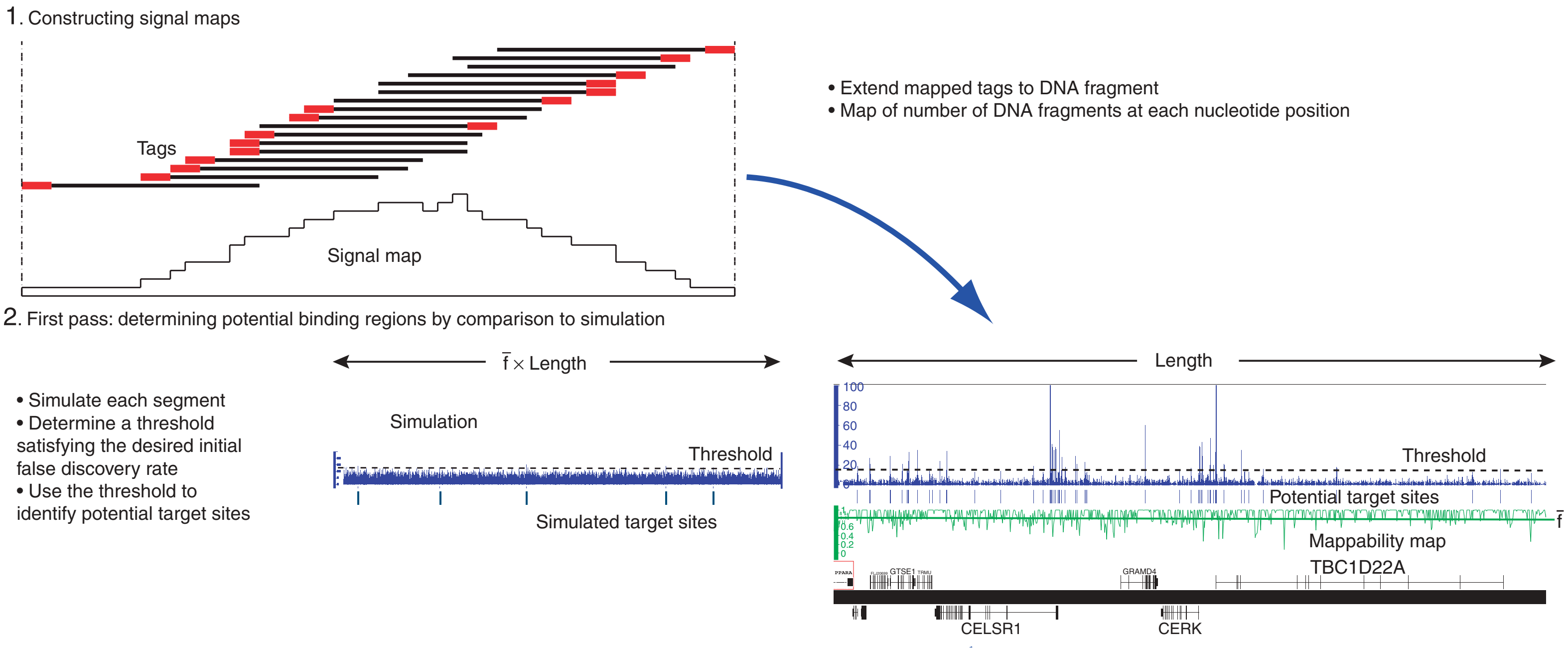
$n_i = k_{1i} + k_{2i}$

$k_{1i} \mid n_i \sim \text{Binom}(n_i, p_0)$

Consider mappability: PeakSeq

Rozowsky et al. (2009) *NBT*

- First round analysis: detect possible peak regions by identifying threshold considering mappability:
  - Cut genome into segment (L=1Mb). Within each segment, the same number of reads are permuted in a region of *f* × *Length*, where *f* is the proportion of mappable bases in the segment.



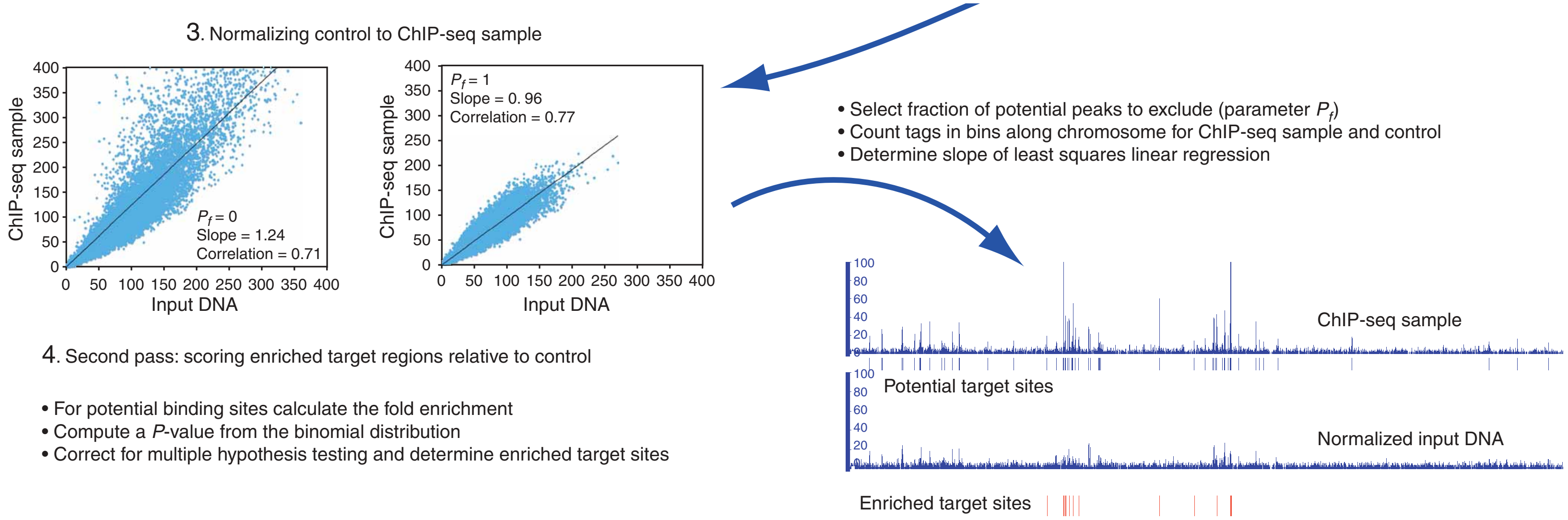
1. Constructing signal maps

- Extend mapped tags to DNA fragment
- Map of number of DNA fragments at each nucleotide position

2. First pass: determining potential binding regions by comparison to simulation

- Simulate each segment
- Determine a threshold satisfying the desired initial false discovery rate
- Use the threshold to identify potential target sites

- Second round analysis:
  - Normalize data by counts in background regions.
  - Test significance of the peaks identified in first round by comparing the total count in peak region with control data, using binomial p-value, with Benjamini-Hochberg correction.



3. Normalizing control to ChIP-seq sample

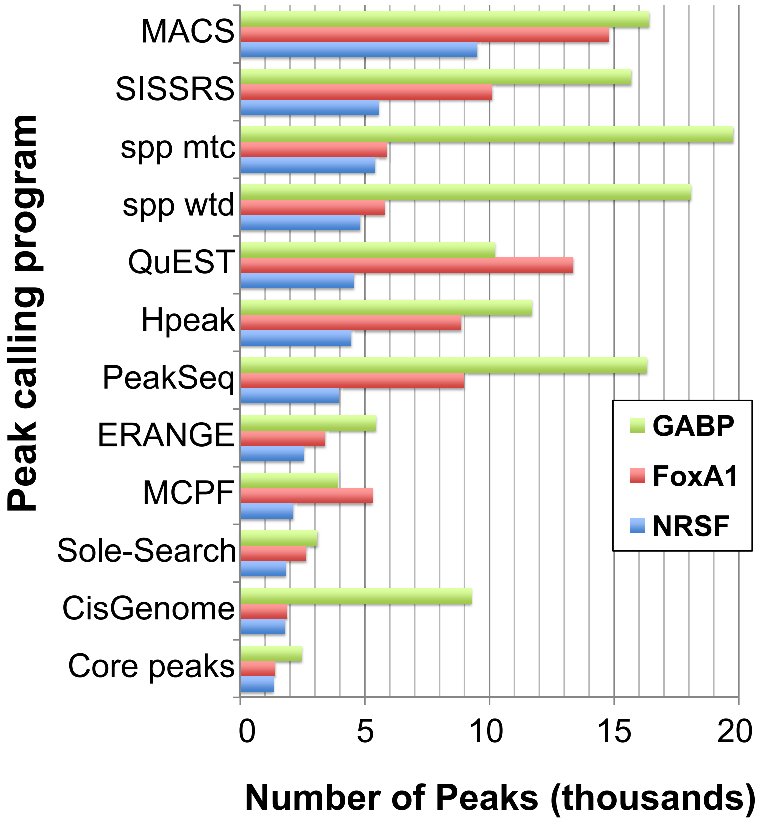
- Select fraction of potential peaks to exclude (parameter P_f)
- Count tags in bins along chromosome for ChIP-seq sample and control
- Determine slope of least squares linear regression

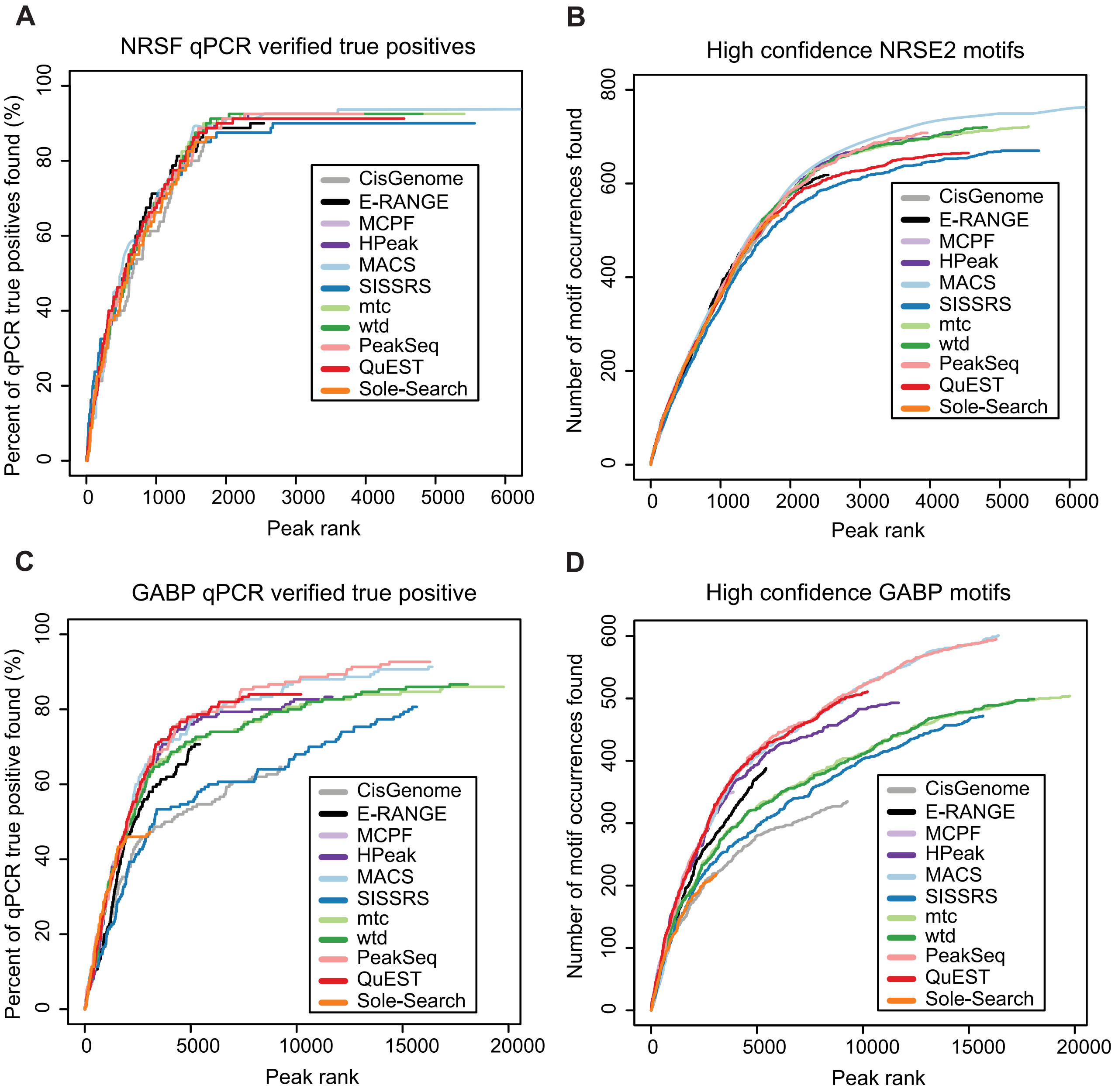
4. Second pass: scoring enriched target regions relative to control

- For potential binding sites calculate the fold enrichment
- Compute a *P*-value from the binomial distribution
- Correct for multiple hypothesis testing and determine enriched target sites

Comparing peak calling algorithms

- Wilbanks et al. (2010) *PloS One*
- Laajala et al. (2009) *BMC Genomics*



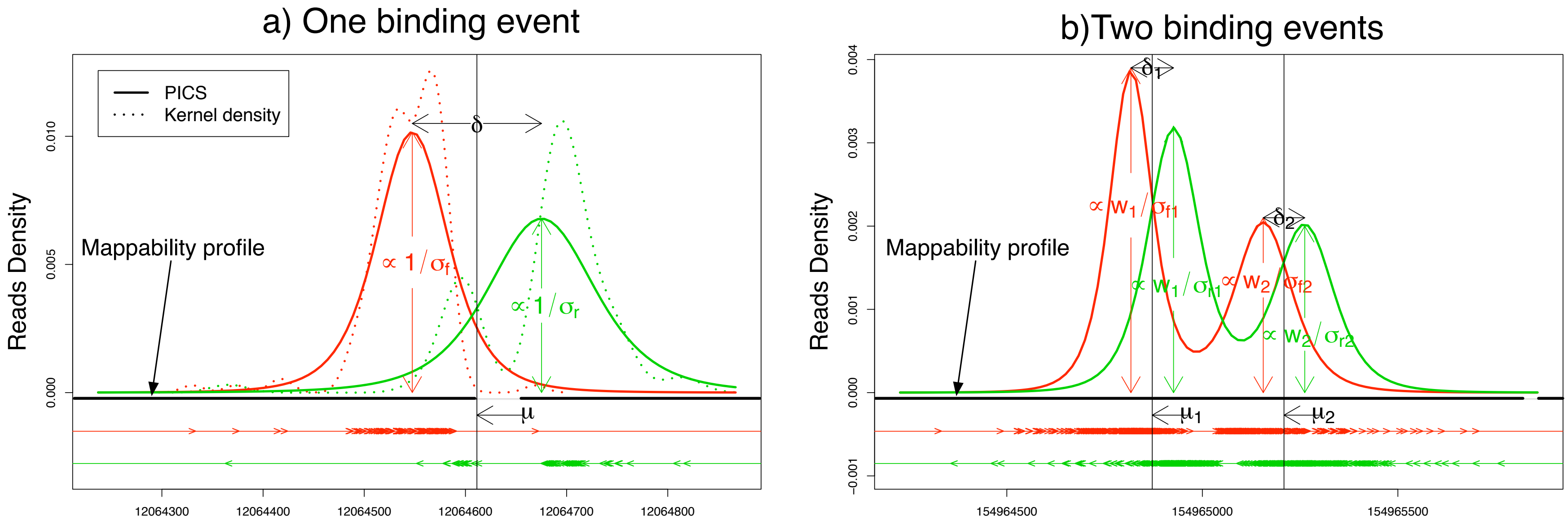

A
NRSF qPCR verified true positives
Percent of qPCR true positives found (%)
0
20
40
60
80
100
0
1000
2000
3000
4000
5000
6000
Peak rank
CisGenome
E-RANGE
MCPF
HPeak
MACS
SISSRS
mtc
wtd
PeakSeq
QuEST
Sole-Search
B
High confidence NRSE2 motifs
Number of motif occurrences found
0
200
400
600
800
0
1000
2000
3000
4000
5000
6000
Peak rank
CisGenome
E-RANGE
MCPF
HPeak
MACS
SISSRS
mtc
wtd
PeakSeq
QuEST
Sole-Search
C
GABP qPCR verified true positive
Percent of qPCR true positive found (%)
0
20
40
60
80
100
0
5000
10000
15000
20000
Peak rank
CisGenome
E-RANGE
MCPF
HPeak
MACS
SISSRS
mtc
wtd
PeakSeq
QuEST
Sole-Search
D
High confidence GABP motifs
Number of motif occurrences found
0
100
200
300
400
500
600
0
5000
10000
15000
20000
Peak rank
CisGenome
E-RANGE
MCPF
HPeak
MACS
SISSRS
mtc
wtd
PeakSeq
QuEST
Sole-Search

Another class of approach: modeling the read locations

- Regions with more reads clustered tend to be binding sites.
- This is similar to using binned read counts.
- Reads mapped to forward/reverse strands are considered separately.
- Peak shape can be incorporated.

PICS: Probabilistic Inference for ChIP-seq
Zhang *et al.* 2010 *Biometrics*

- Use shifted t-distributions to model peak shape.
- Can deal with the clustering of multiple peaks in a small region.
- A two step approach:
  - Roughly locate the candidate regions.
  - Fit the model at each candidate region and assign a score.
- EM algorithm for estimating parameters.
- Computationally very intensive.
- R/Bioconductor package available.



a) One binding event

b)Two binding events

$$f_i \sim \sum_{k=1}^{K} w_k t_4\left(\mu_{fk}, \sigma^2_{fk}\right) \overset{d}{=} g_f(f_i|\boldsymbol{w}, \boldsymbol{\mu}, \boldsymbol{\delta}, \boldsymbol{\sigma}_f)$$

$$r_j \sim \sum_{k=1}^{K} w_k t_4\left(\mu_{rk}, \sigma^2_{rk}\right) \overset{d}{=} g_r(r_j|\boldsymbol{w}, \boldsymbol{\mu}, \boldsymbol{\delta}, \boldsymbol{\sigma}_r)$$

GPS (Genome Positioning System)

Guo *et al.* 2010, *Bioinformatics*

- Part of GEM (Genome wide Event finding and Motif discovery) software suite.
- The general idea is very similar to PICS.
- Use non-parametric distribution to model the peak shape.
- Estimation of peak shape and peak detection are iterated until convergence.
- Written in Java, runs in command line.

Use GPS

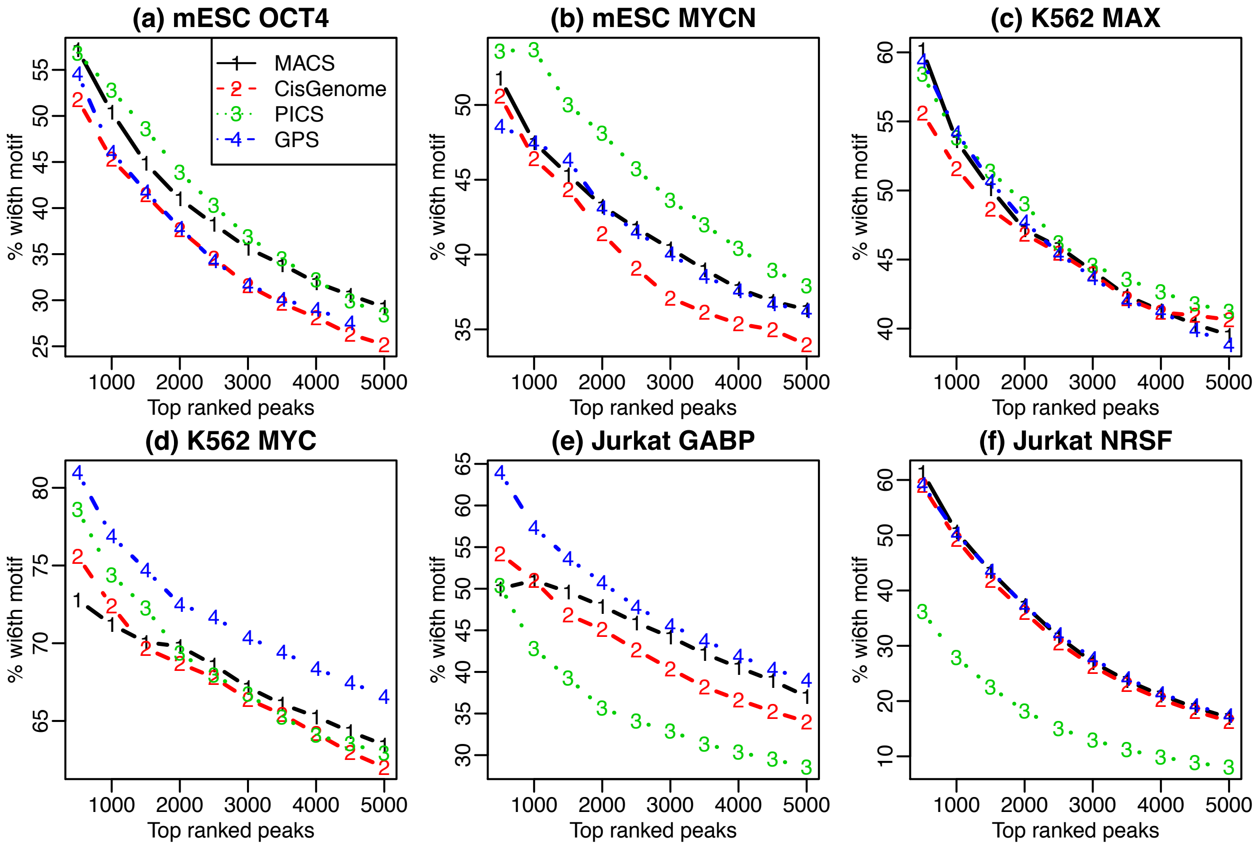
- Run following command:

```
java -Xmx1G -jar gps.jar --g mm8.info --d
Read_Distribution_default.txt --expt IP.bed
--ctrl control.bed --f BED --out result
```

- It’s much slower than MACS or CisGenome.

A little more comparison

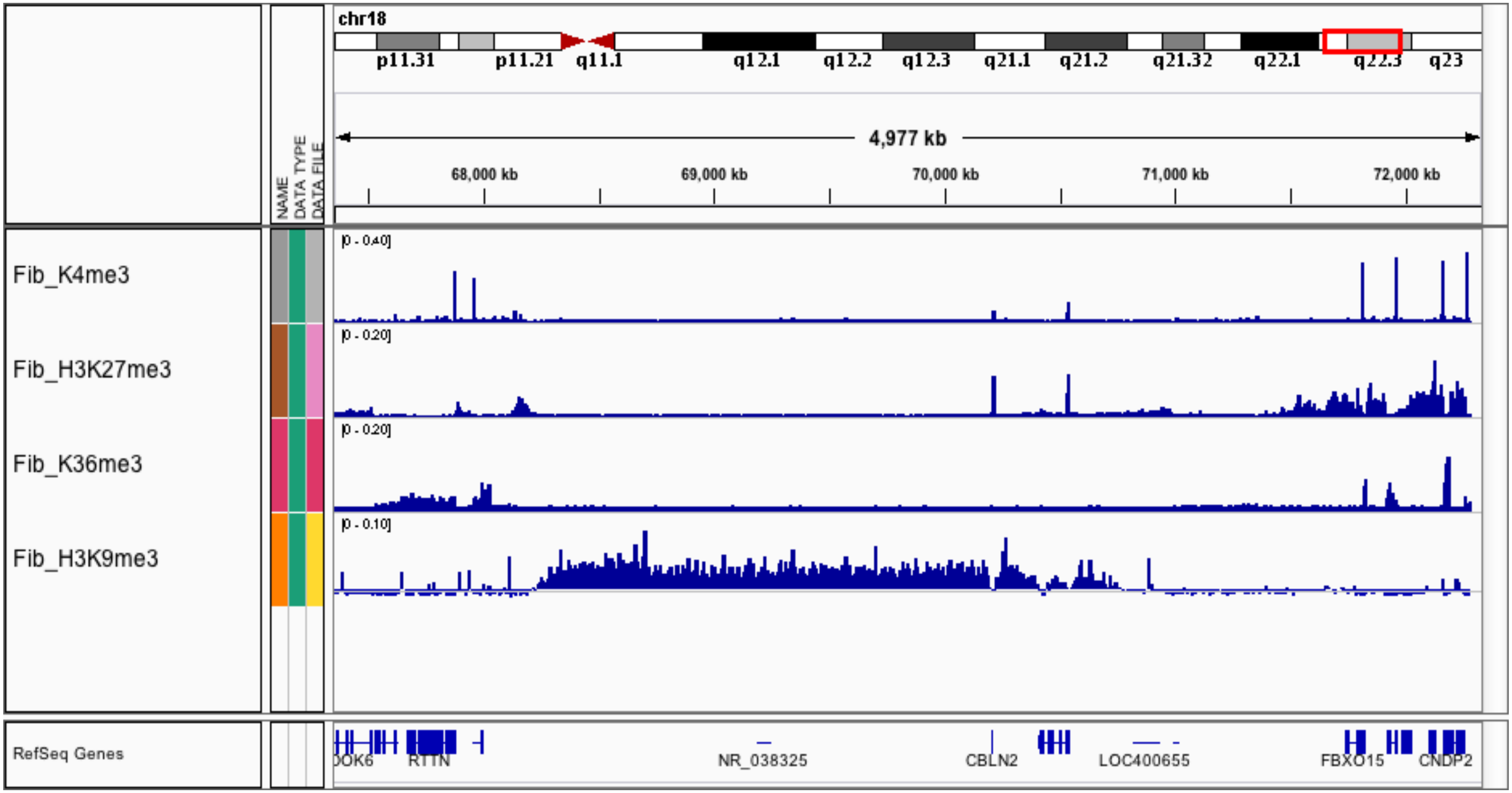
- I found that using peak shapes helps. GPS tend to perform better. PICS seems not stable.



ChIP-seq for histone modification

- Histone modifications have various patterns.
  - Some are similar to protein binding data, e.g., with tall, sharp peaks: H3K4.
  - Some have wide (mega-bp) “blocks”: H3k9.
  - Some are variable, with both peaks and blocks: H3k27me3, H3k36me3.

Histone modification ChIP-seq data



Peak/block calling from histone ChIP-seq

- Use the software developed for TF data:
  - Works fine for some data (K4, K27, K36).
  - Not ideal for K9: it tends to separate a long block into smaller pieces.
- Existing methods based on: smoothing, HMM, wavelet, etc.
- Method for detecting blocks is relatively under-developed and under-tested:
  - ENCODE is evaluating existing methods.

Complications in histone peak/block calling

- Smoothing-based method:
  - Long block requires bigger smoothing span, which hurts boundary detection.
  - Data with mixed peak/block (K27me3, K36me3) requires varied span: adaptive fitting is computationally infeasible.
- HMM based method:
  - Tend to over fit. Sometimes need to manually specify transition matrix.

Available methods/software for histone data peak calling

- MACS2
- BCP (Bayesian change point caller)
- SICER
- RSEG
- UW Hotspot
- BroadPeak
- mosaicsHMM
- WaveSeq
- ZINBA
- …

Summary for ChIP-seq peak/block calling

- Detect regions with reads enriched.
- Control sample is important.
- Incorporate some special characteristics of the data improves results.
- Calling blocks (long peaks) is harder.
- Many software available.

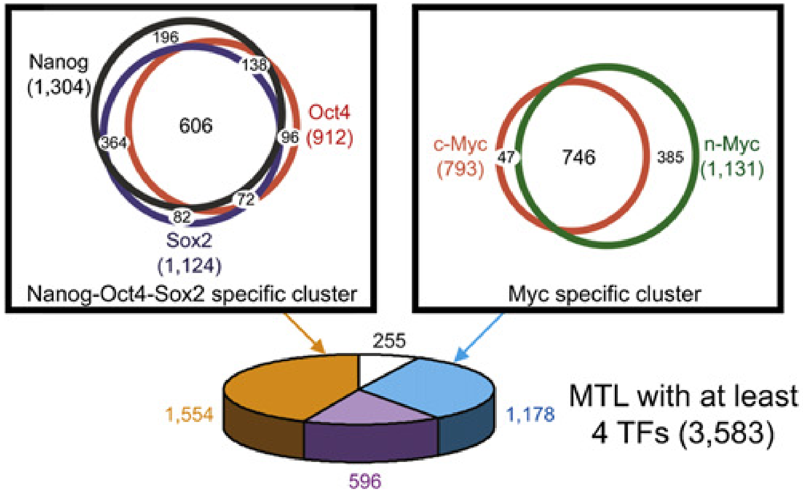
Downstream analysis after peak/block calling

After peak/block calling

- Compare results among different samples:
  - Presence/absence of peaks.
  - Differential binding.
  - Look for Combinatory patterns.
- Compare results with other type of data:
  - Correlate TF binding with gene expressions from RNA-seq or DNA methylation from BS-seq.

Comparison of multiple ChIP-seq

- It's important to understand the co-occurrence patterns of different TF bindings and/or histone modifications.
- Post hoc methods: look at overlaps of peaks and represent by Venn Diagram.
  - This can be done using different tools: ***BEDtools***, ***Bioconductor***, etc.
  - We will practice in the lab.



Differential binding (DB) analysis

- Problems for the overlapping analysis are:
  - Completely ignores the quantitative differences of peaks.
  - Highly dependent on the thresholds for defining peaks.
- More desirable: quantitative comparison to detect differential protein binding or histone modification (referred to as “DB analysis”).
- Typical DB analysis procedure:
  - Call peaks from individual dataset.
  - Union the called peaks to form candidate regions.
  - Hypothesis testing for each candidate region.

Complications in DB analysis

- Different backgrounds: for example, chromatin structures affect the sequencing efficiency.
- Signal to noise ratios (SNR) from different experiments:
  - Biological: sample with less peak will have taller peaks.
  - Technical: qualities of the experiments are different.
- To summarize:
  - DB is more complicated than RNA-seq DE problem.
  - Methods are relatively under-developed.

Existing methods for DB analysis

- Normalize data first, then compare:
  - **MAnorm** (Shao *et al.* 2012, *Genome Biology*): normalization based on MA plot of counts from two conditions, then use normalized "M" values to rank differential peaks.
  - **ChIPnorm** (Nair *et al.* 2012, *PLoS One*): quantile normalization for each dataset, then define differential peak based on normalized IP differences.
- Based on RNA-seq DE methods:
  - **DBChIP**: Liang *et al.* (2012) Bioinformatics.
  - **DiffBind**: A Bioconductor package.
- Model the differences of data from two IP sample:
  - **DIME** (Taslim *et al.* 2009, 2011, *Bioinformatics*): finite mixture model on differences of normalized IP counts.
  - **ChIPDiff** (Xu et al. 2008, Bioinformatics): HMM on differences of normalized IP counts between two groups.

Review

- NGS provides cost-effective ways for various aspects of genomic research.
- ChIP-seq is a type of NGS for genome-wide regional analysis: detect protein binding or histone modification regions.
- Main goal of ChIP-seq data analysis is “peak/block” calling.
  - Many software available, based on smoothing or HMM.
  - Block calling is harder.
- Comparison of ChIP-seq signals (differential binding analysis) is still an open problem.