

Module 9: ChIP-sequencing

Presented by:

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Based on materials by:
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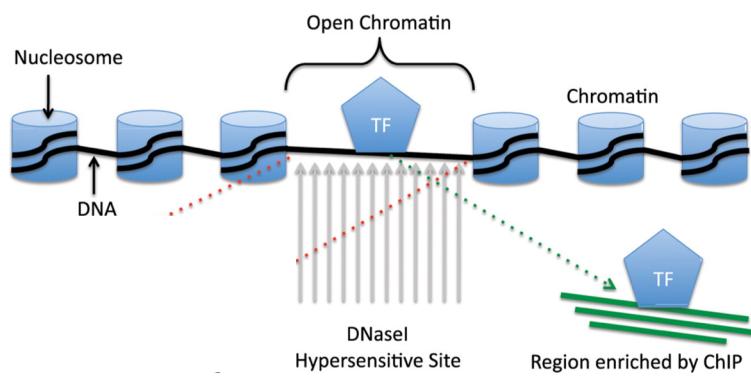
Next Generation Sequencing Bioinformatics Course
18-22 January 2021 - Santiago - Chile



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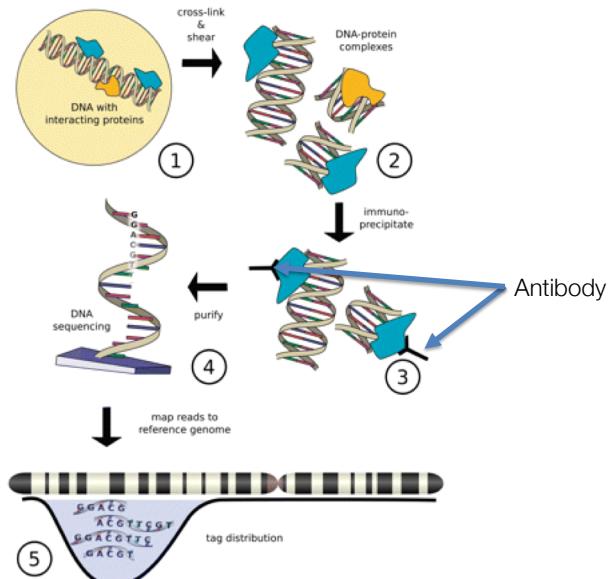
WELLCOMBE GENOME CAMPUS
CONNECTING SCIENCE
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Epigenetics/ChIP in one slide

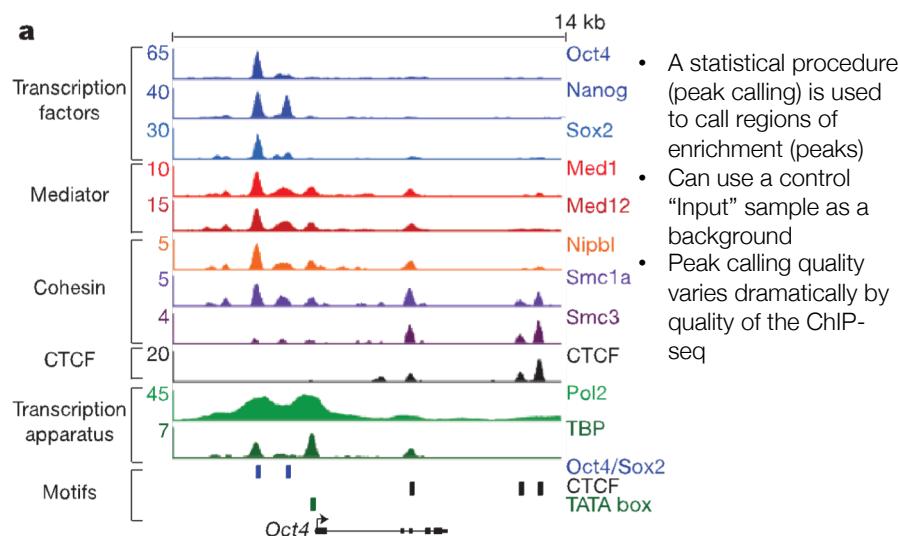


- Regulation of transcription involves interaction of protein and DNA

How does ChIP-seq work?



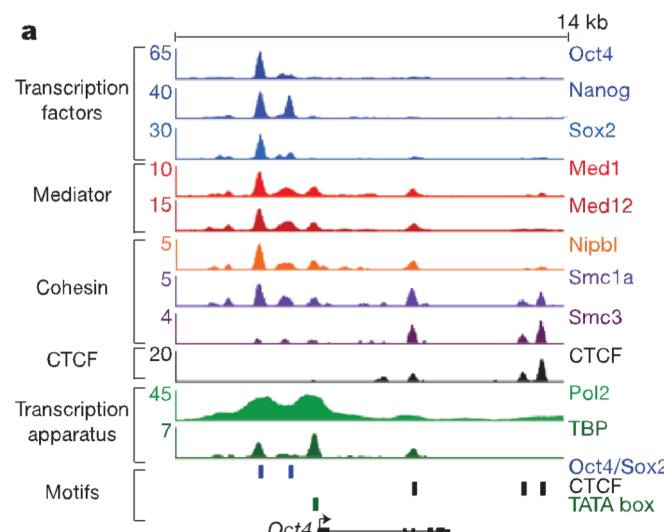
What does ChIP-seq look like?



Applications of ChIP-seq

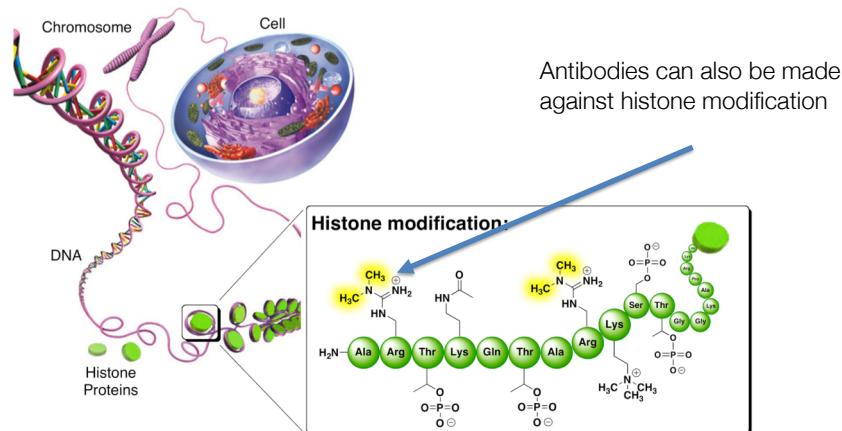
- ChIP-seq is one of the most commonly used approaches for identifying gene regulatory regions
- Two common types:
 1. Transcription factors
 2. Histone modifications

ChIP-seq for transcription factors



- Each of these TFs requires a high quality, ChIP-grade anti-body
- Most antibodies (~60%) are not good enough for ChIP-seq

Histone modifications



Histone mark cheat sheet

Histone mark	Candidate State	Interpretation
H3K9me2,3	-	Silenced genes
H3K27me3	Inactive/poised promoter, polycomb repressed	Downregulation of nearby genes
H3K36me3	Transcriptional transition	Actively transcribed gene bodies.
H4K20me1	Transcriptional transition	Transcriptional activation
H3K4me1,2,3	Strong enhancer	Promoter of active genes
H3K27ac	Active promoter/strong enhancer	Active transcription
H3K9ac	Active promoter	Switch from transcription initiation to elongation.

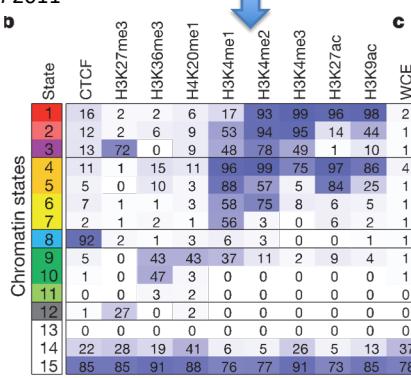
EPIGENETIC JARGON CHEAT - SHEET

Regulatory Element	Meaning
Promoter	DNA Sequence (100-1kb), initial secure binding site for: RNA Pol complex Transfacs Adjacent regulated gene, defined relative to TSS. Poised: simultaneous activation/repressive histone mods.
Enhancer/Silencer	DNA Seq (50-1.5kb), bound by transfacs (<i>activator / repressor</i>) Can act on gene up to 1Mb away: DNA folding brings it close to promoter. Enhancer: Bound by activator, which interacts with complex initiating transcription. Silencer: bound by repressor, which interferes with GTF assembly.
Insulator	DNA, 300-2kb, Block enhancers from acting on promoters: positioned between enhancer and promoter, form chromatin-loop domains.
Polycomb-repressed	Polycomb – group proteins actively remodel chromatin to silence genes.

The histone code

Then: go back and ask what fraction of classified regions contain peaks of a given type.

Ernst et al 2011



First: create these categories by applying HMM classifying stretches of genome to combined peak data:
9 cell lines x 9 chromatin marks.
Apply functional interpretation after categories are created.

(NH) ⁺	Candidate state annotation
Active promoter	
Weak promoter	
Inactive/poised promoter	
Strong enhancer	
Strong enhancer	
Weak/poised enhancer	
Weak/poised enhancer	
Insulator	
Transcriptional transition	
Transcriptional elongation	
Weak transcribed	
Polycomb repressed	
Heterochrom; low signal	
Repetitive/CNV	
Repetitive/CNV	

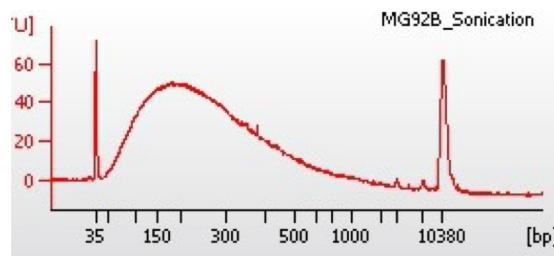
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H3K27ac	Active promoter/strong enhancer	Active transcription
H3K9ac	Active promoter	Switch from transcription initiation to elongation.

ChIP-seq experimental considerations

- Antibody quality: 60% of antibodies not high enough quality
- Numbers of cells: 2-3M recommended, more for TFs (5-10M)
- Crosslinking time: ~10 mins
- Shearing

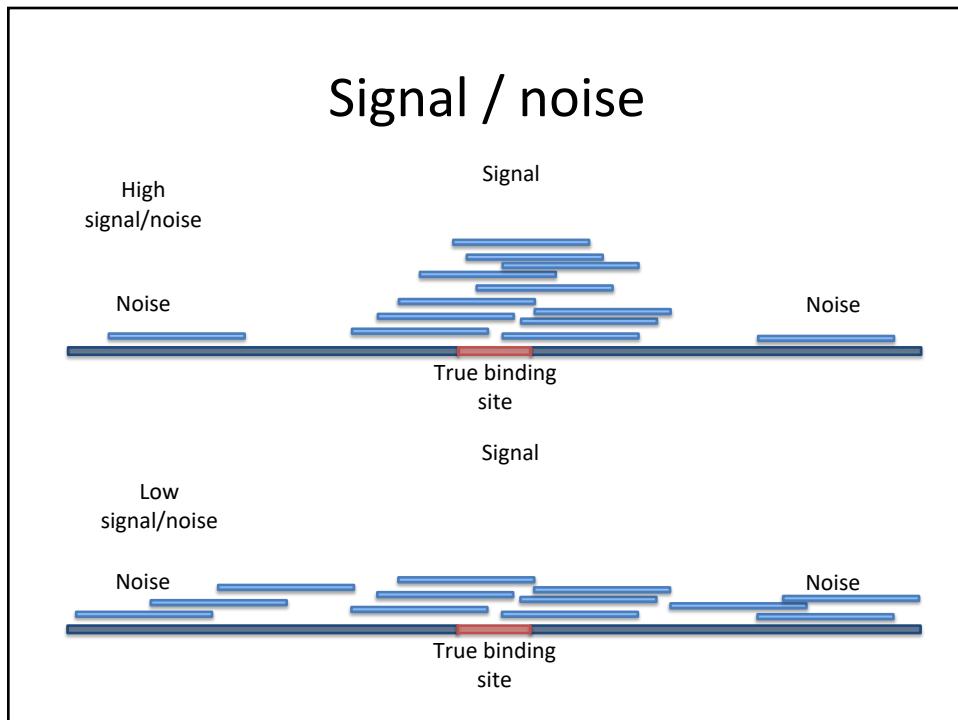
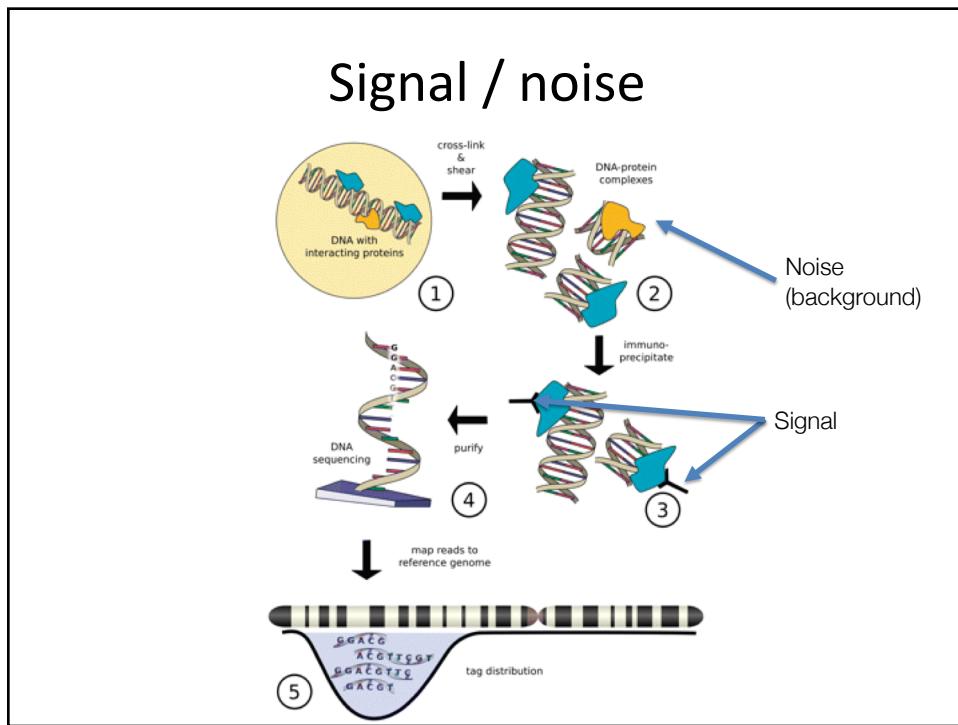
Shearing

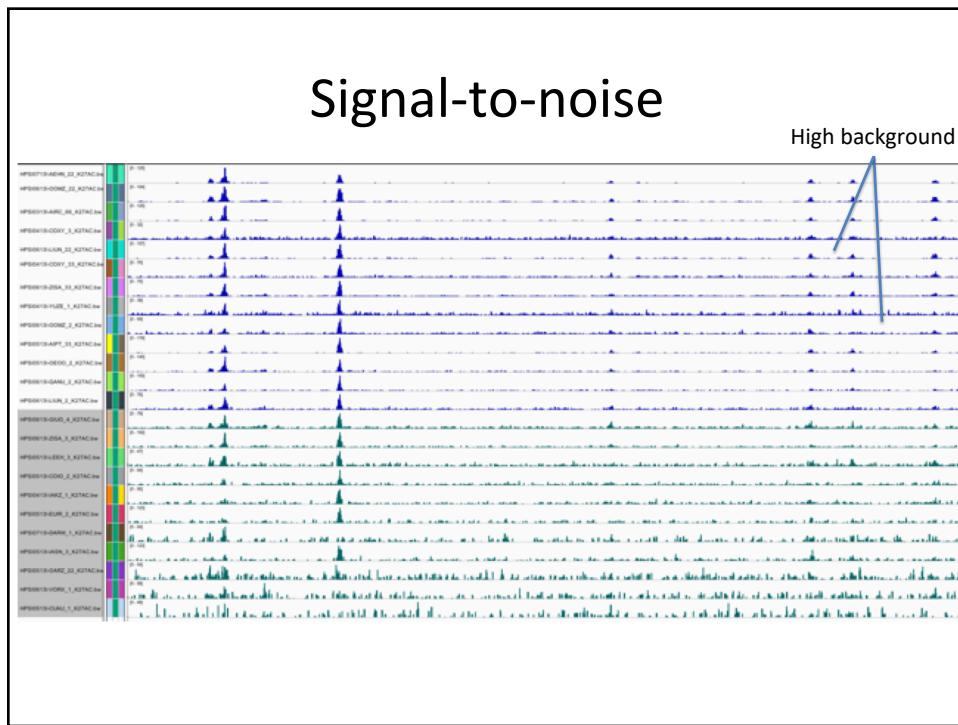


- Aim for fragments in 150-400bp range
- Efficiency varies by cell type
- Optimise by varying number of shearing cycles
- Run input samples on Bioanalyser to check efficiency

ChIP-seq technical issues

1. Signal / noise: Does my antibody work?
2. Library complexity: Did I have enough starting material?





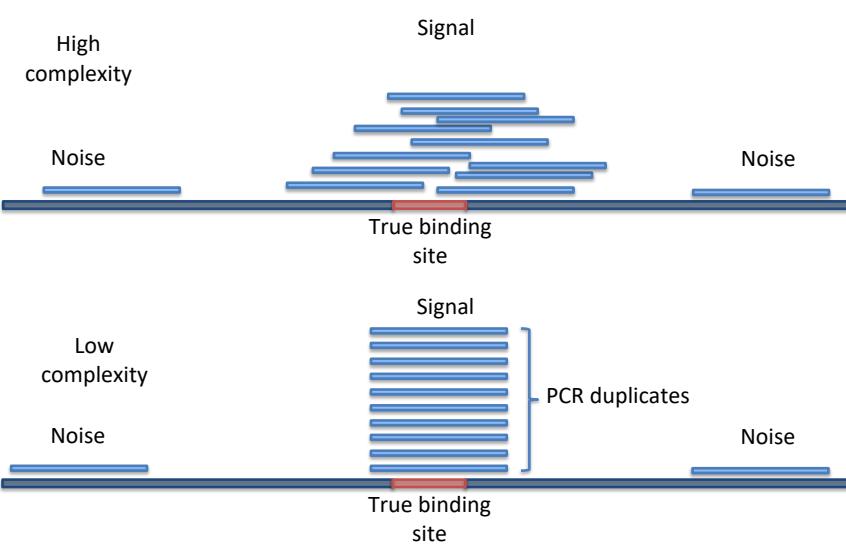
FRIP

- Fragments In Peaks
- # Fragments found in peaks / Total # fragments
- >1%

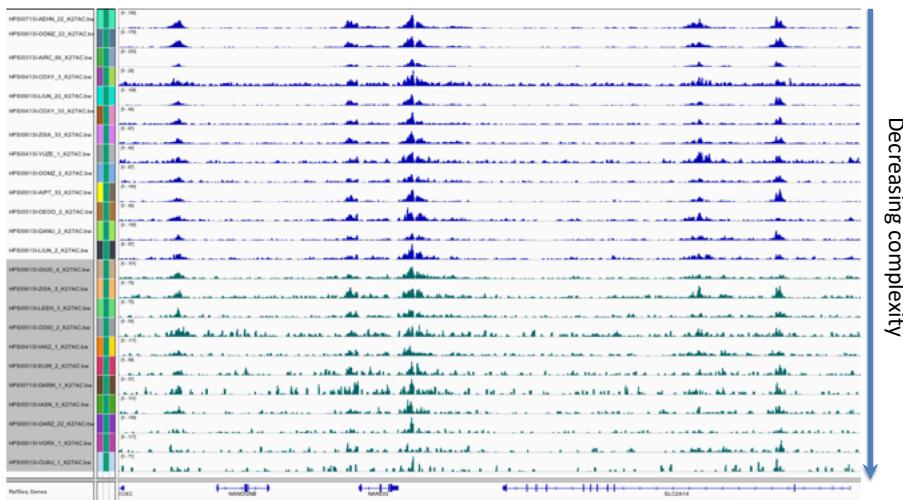
Library complexity

- Problem: Not enough starting material
 - Not enough cells
 - Antibody efficiency
- More PCR required

Library complexity



Library complexity



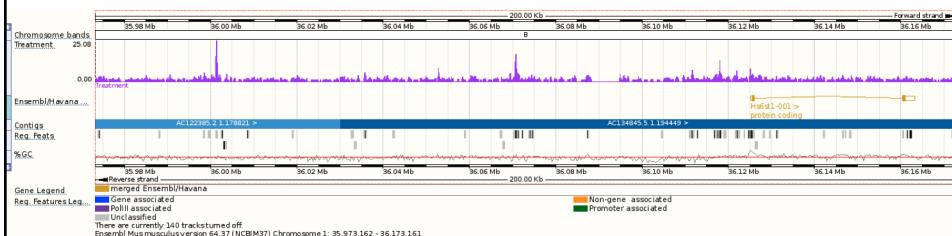
Nonredundant fraction

- # unique fragments positions / total # fragments
- >0.8

Basic analysis of ChIP-seq

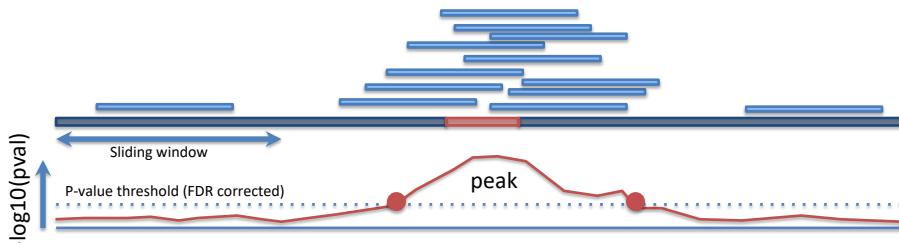
1. Read alignment
2. Visualisation
3. Peak calling
 - Peak annotation (mapping peaks to genes etc)
 - Motif analysis
4. Differential binding
 - Case / control
 - Naïve / stimulated

Visualisation in a genome browser



- Convert mapped reads to “signal” – e.g. read depth at each bp or in windows
- BAM files to e.g. wig, bedgraph
- IGV, ensembl, UCSC

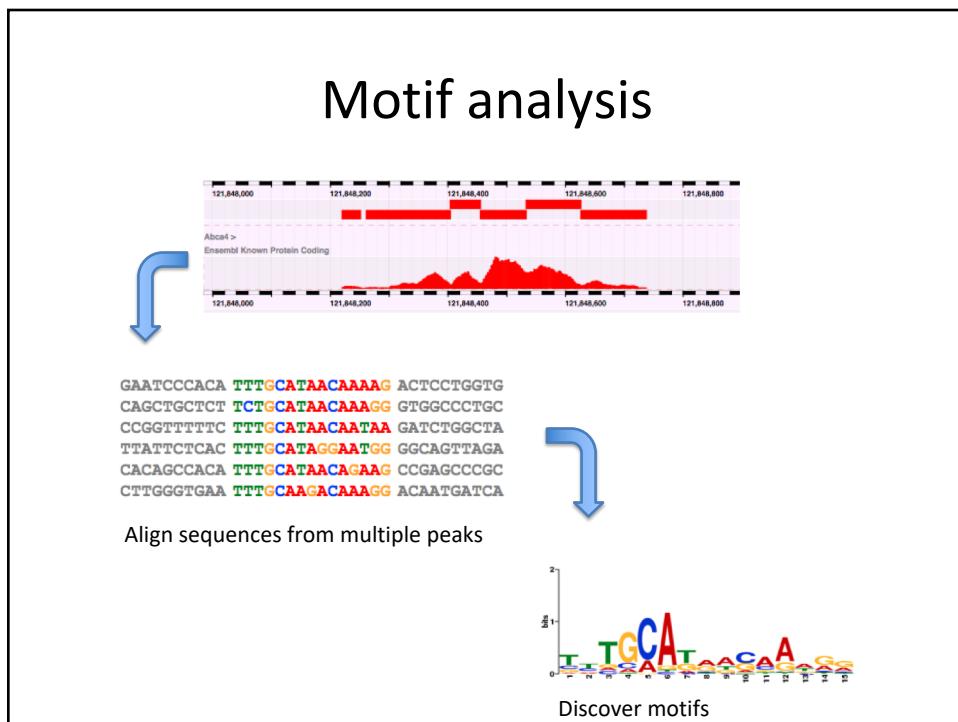
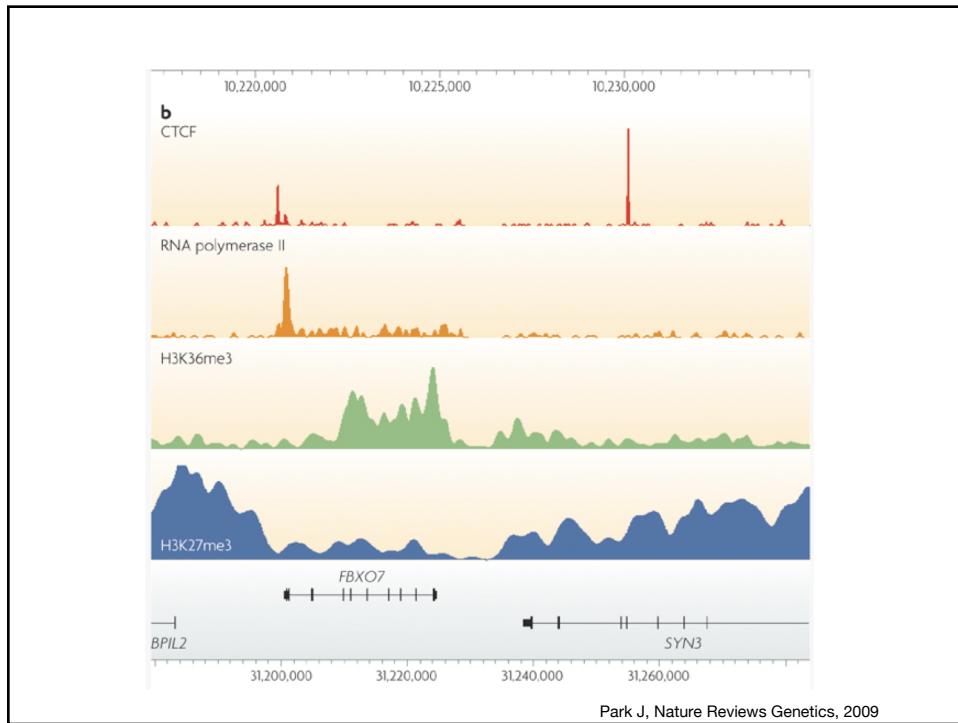
Peak calling



- Observed counts
- Expected counts
- Poisson test: $p\text{-value} = \text{prob}(\text{observing frag count at least as extreme under null})$

Peak calling challenges

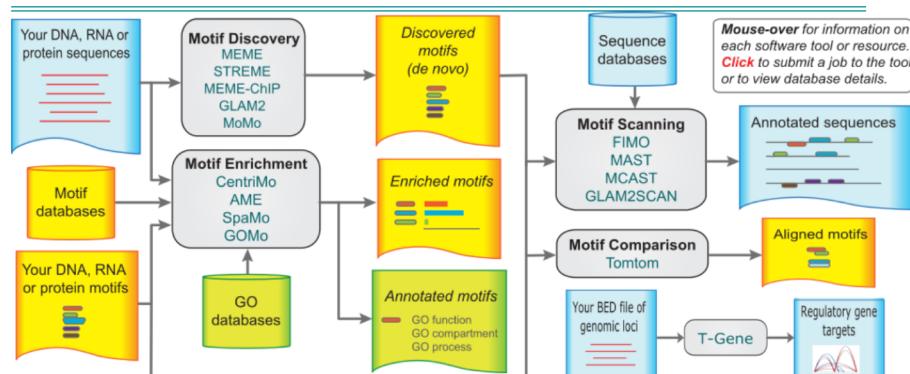
- What's expected?
 - Treatment sample (with antibody)
 - Input sample (no antibody)
- Replicates
 - Yes! (min 2, more = better)
- Peak sizes
 - These are variable: small for TFs, large for some Histone mods, and for Pol2 etc.



Motif analysis tools

The MEME Suite

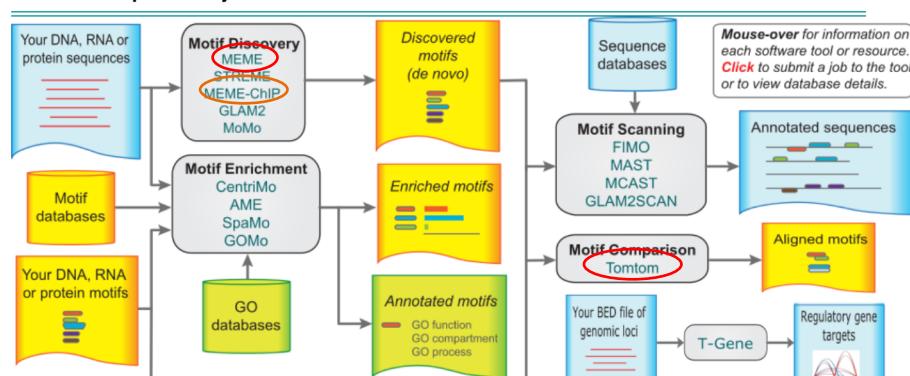
Motif-based sequence analysis tools



Motif analysis tools

The MEME Suite

Motif-based sequence analysis tools



Motif analysis tools



HOMER

Software for motif discovery and next-gen sequencing analysis

Homer de novo Motif Results

Knows: Motif Enrichment Results
Gene Ontology Enrichment Results
If Homer is having trouble matching a motif to a known motif, try copy/pasting the matrix file into STAMP.
View the results from the previous analysis using results HOMER | Description of Results | Tips
Total target sequences = 1351
Total background sequences = 19001
* = possible false positive

Rank/Motif	Motif	P-value	log P-value	% of Targets	% of Background	STD(Bg/ STD)	Best Match Details
1	ACAUUCCG	1e-116	-5.584e+02	55.29%	24.70%	1843.3bp (1700.0bp)	hsa-miR-206 MIMAT0000462 Homo sapiens miR-206 Targets (miRBase) More Information Similar Motif Found
2	AGUAUAGAC	1e-41	-9.537e+02	68.39%	49.40%	1877.0bp (1667.9bp)	hsa-miR-485-3p MIMAT0002176 Homo sapiens miR-485-3p Targets (miRBase) More Information Similar Motif Found
3	CAUCGACG	1e-28	-6.532e+02	60.92%	45.25%	1651.8bp (1428.1bp)	hsa-miR-181a* MIMAT0000273 Homo sapiens miR-181a* Targets (miRBase) More Information Similar Motif Found

