ChIP-Seq Data Analysis 1

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BIOS 6660

Spring 2019

Overview From Last Time

- Walked through RNA-seq pre-processing on yampa
- Many independent scripts
- Good to have a main document which shows which scripts you used
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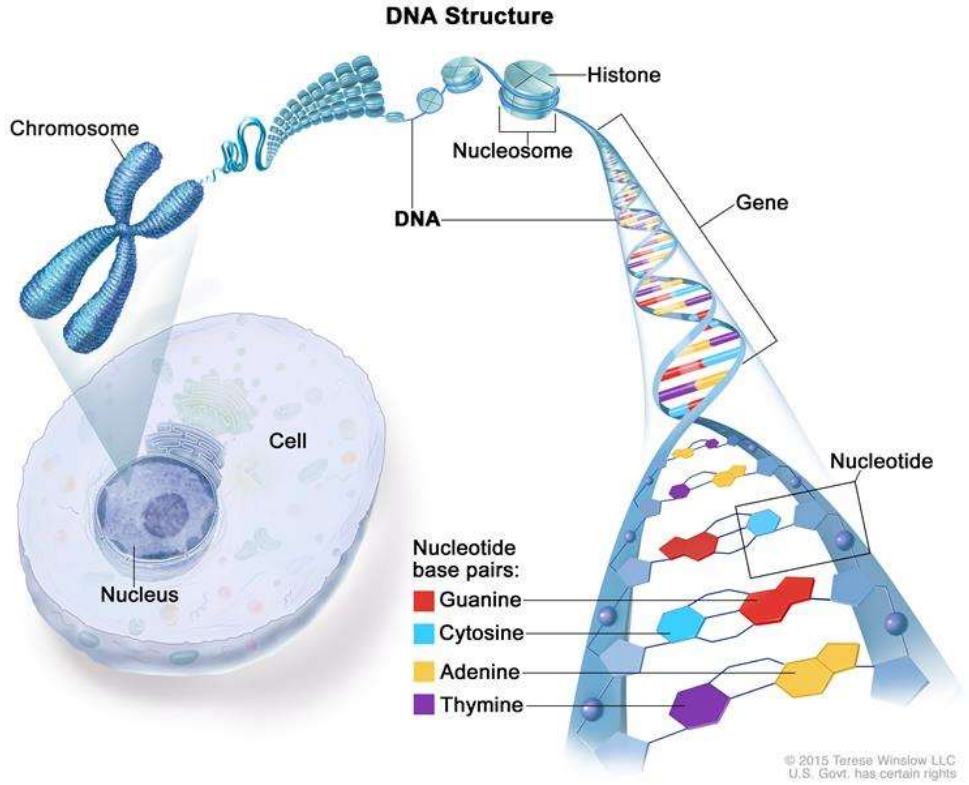
Chromosomes

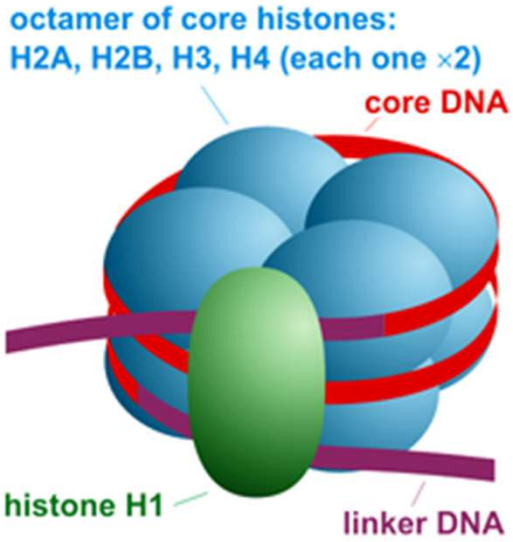
- How DNA is stored in the cell
- >3 billion bases, need to fit in a small space
- DNA is wrapped around proteins called histones which coil into chromosomes
- Chromatin is the DNA and proteins making up the chromosomes

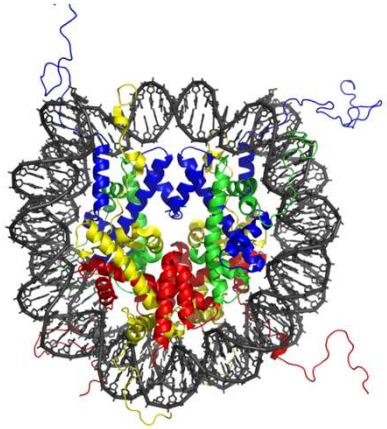


Source: National Cancer Institute

Nucleosome

- Basic unit of packaging
- Consists of DNA wrapped around a core of 8 histone proteins
- Compared to a spool
- Nucleosome positions in the genome are not random
- Determines the accessibility of the DNA to regulatory proteins

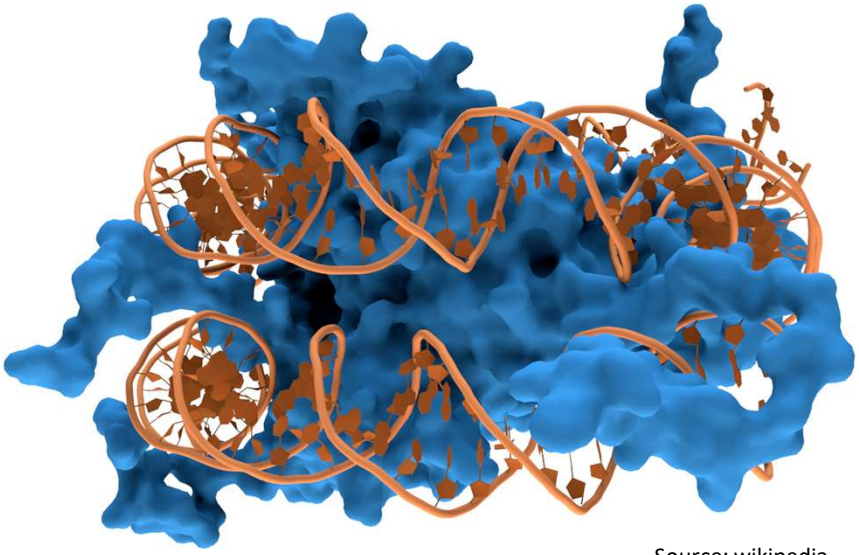




Source: Wikipedia

DNA-Protein Physical Interaction

- Many types of DNA-binding proteins
  - Transcription Factors
    - Regulate gene expression
  - Polymerases
    - Enzymes synthesizes long chains of polymers or nucleic acids
  - Nucleases
    - Enzyme which can cleave DNA
  - Histones
    - Chromosome packaging
- For ChIP-Seq we will be interested in transcription factors and histone binding



Source: wikipedia

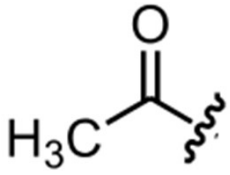
A histone is shown in blue and DNA in gold
3D structure is very complex

Chromatin Remodeling

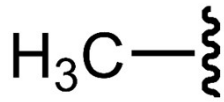
- Dynamic modification of chromatin architecture to allow access of condensed genomic DNA
- Allows regulatory transcription machinery proteins to access DNA
  - regulates gene expression
- 2 main methods:

1. Covalent histone modifications by specific enzymes
   - Histone acetyltransferases (HATs)
   - Deacetylases
   - Methyltransferases
   - Kinases
2. ATP-dependent chromatin remodeling complexes which either move, eject or restructure nucleosomes

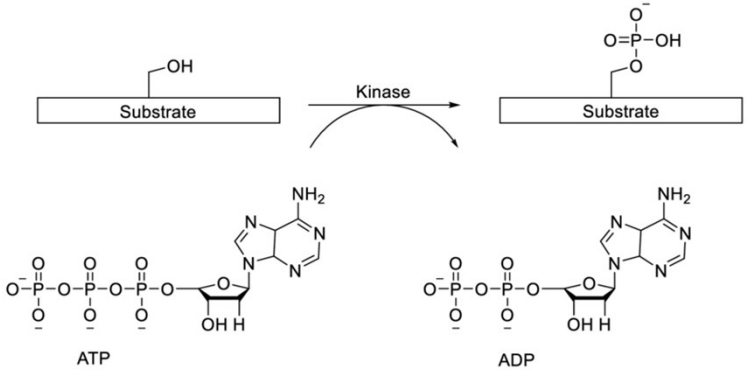
Acetyl group:



Methyl group:



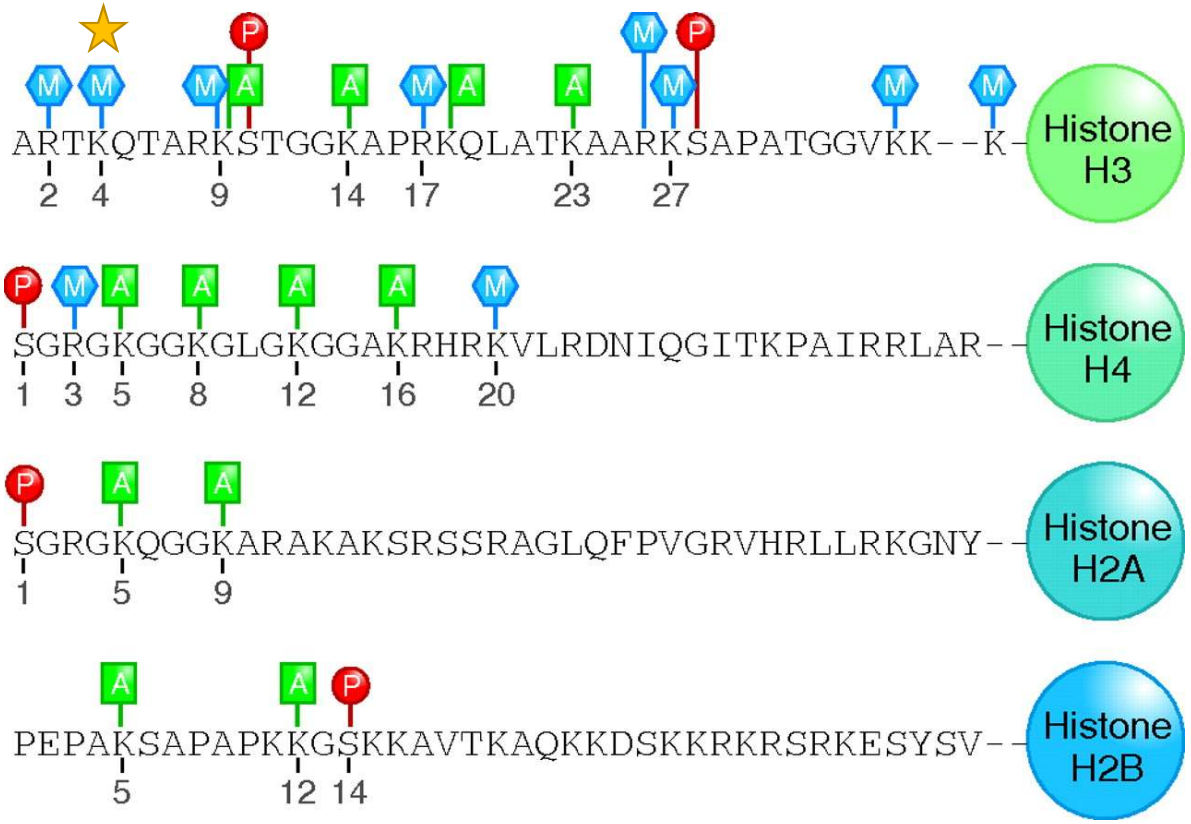
Kinases:



Source: Wikipedia

Histone Modifications

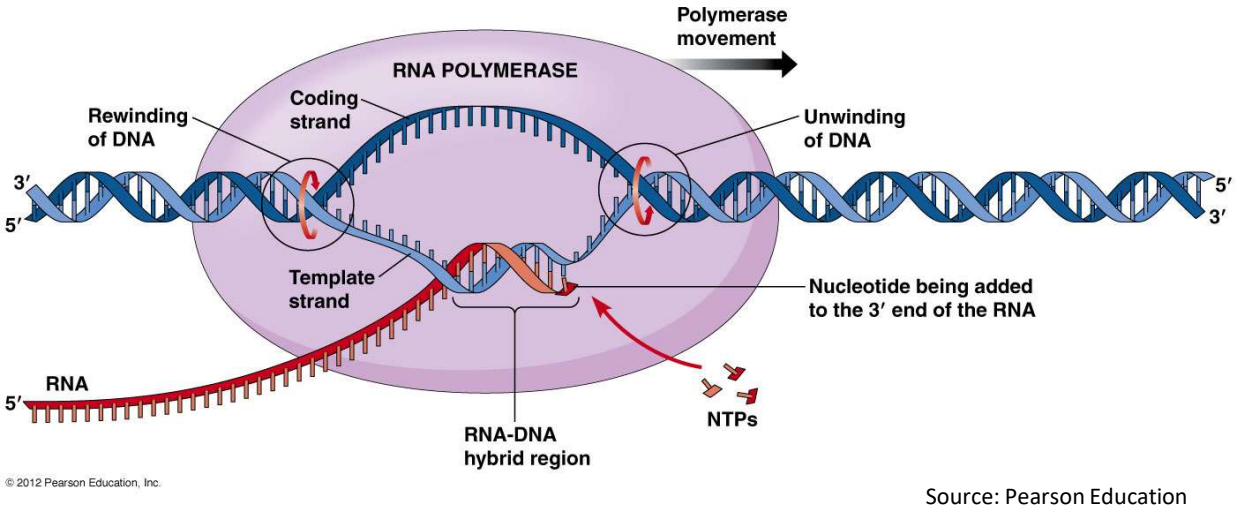
- mono-, di-, or trimethylation (M) at arginine or lysine residues
- acetylation (A) at lysine residues
- phosphorylation (P) at serine residues.
- Some amino acids, such as lysine 9 of histone H3, can be subject to either acetylation or methylation, but not both
- H3 trimethyl K4 is popular to look at



Source: Dressler et al, 2008

Transcription DNA RNA

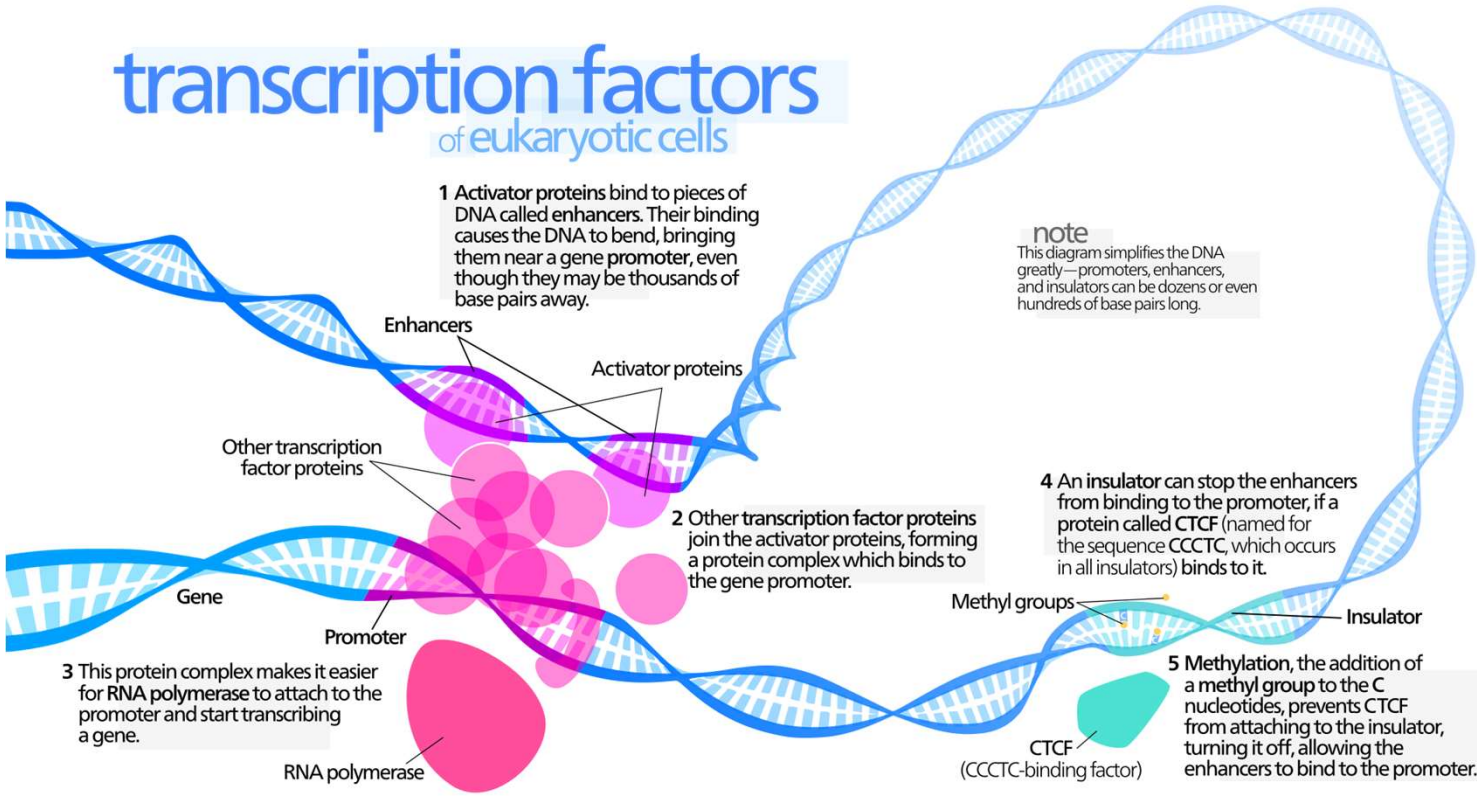
- RNA Polymerase needs to attach to DNA
- But how does it know where to go and bind to?
- Promoter regions
  - ≈100–1,000 bp long right before the transcription start site (TSS)
  - TATA box
- Enhancer Regions
  - ≈ 50–1,500 bp long
  - Can be located up to 1 Mb away
  - always cis-acting



Source: Pearson Education

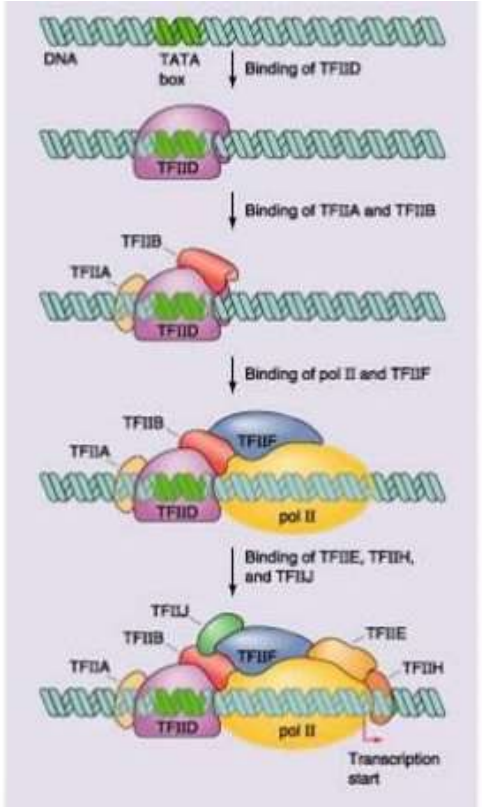
Transcription Factors

- Basal, or general, transcription factors are necessary for RNA polymerase to function at a site of transcription in eukaryotes
  - Considered the most basic set of proteins needed to activate gene transcription
- Bind to either enhancer or promoter regions of DNA adjacent to the genes that they regulate
- Can cause gene to be either up- or down- regulated
- Many mechanisms:
  - Stabilize or block the binding of RNA polymerase to DNA
  - Catalyze acetylation or deacetylation of histones
  - Recruit coactivator or corepressor proteins to the transcription factor DNA complex



Source: wikipedia

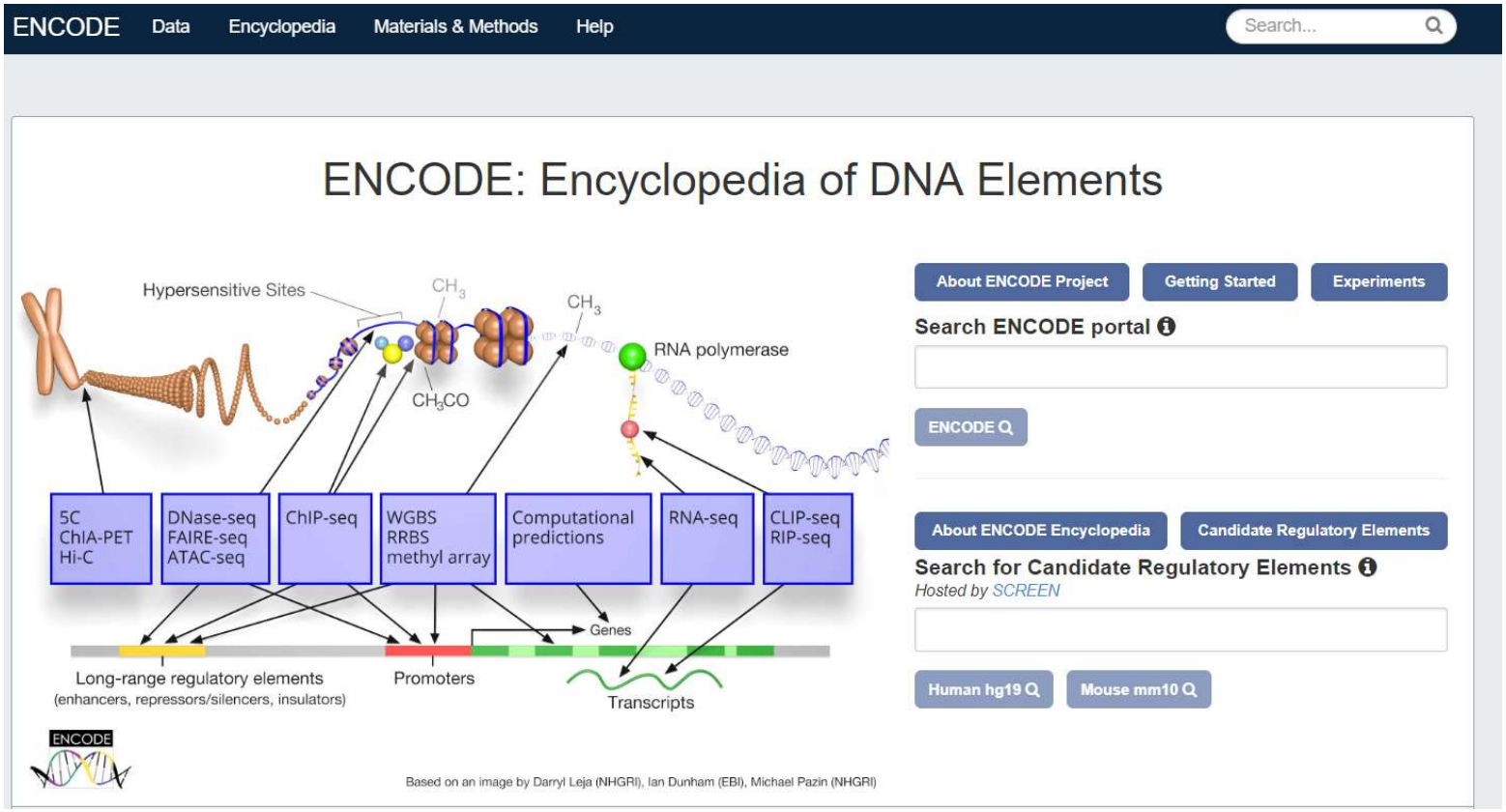
Example of Basal TF



Source: slideServe

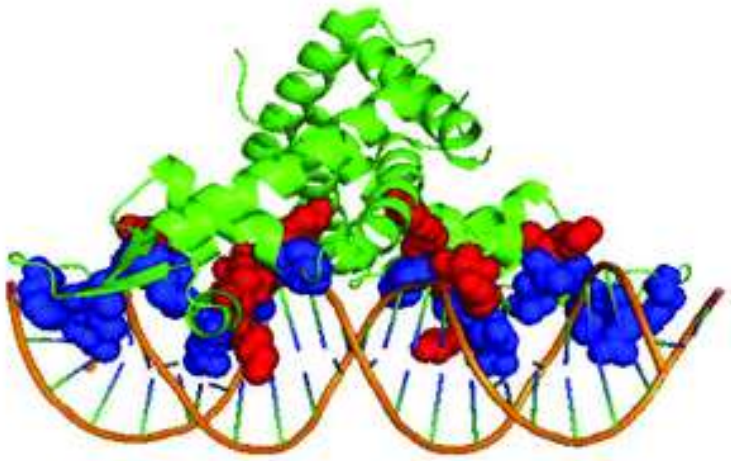
ENCODE

- https://www.encodeproject.org/



ChIP-Seq

1. Chromatin immunoprecipitation (**ChIP**)
2. DNA sequencing (**Seq**)

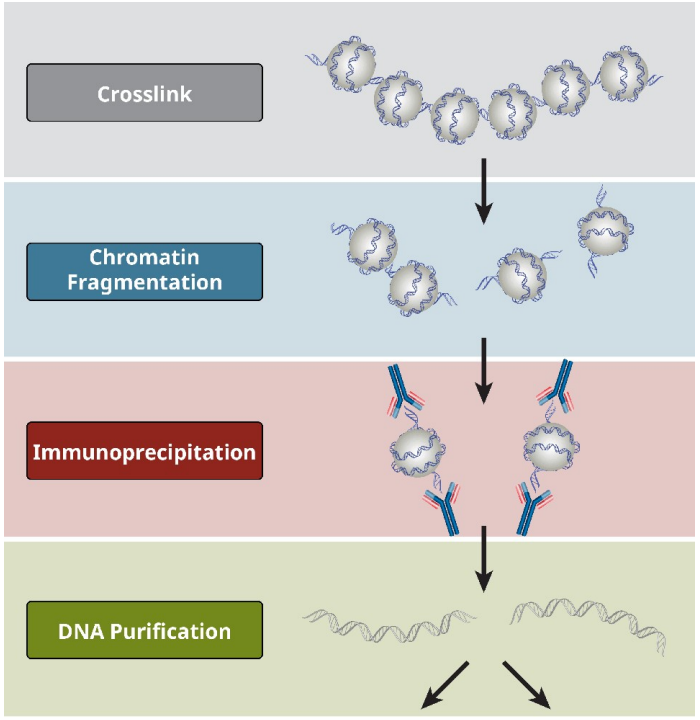


Source: Shen et al, 2017

- **Goal:** identify the binding sites of DNA-associated proteins.

Chromatin Immunoprecipitation (Steps 1&2)

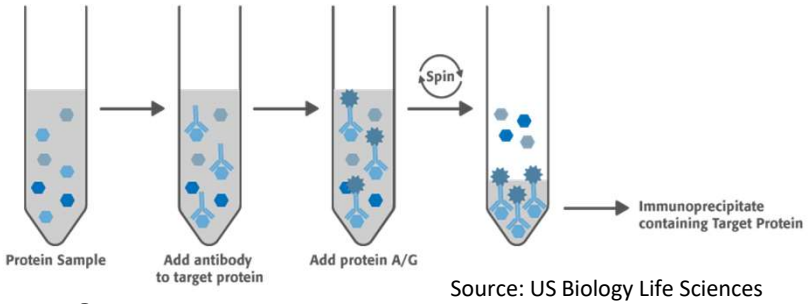
1. Crosslink DNA to protein so stays stable OR Native Approach
   - Crosslink using formaldehyde or UV light
   - Use crosslinking when looking at TF
   - Use native state when looking at histone modifications
2. Fragment
   - Sonication will shear chromatin



Source: Cell Signaling Technologies

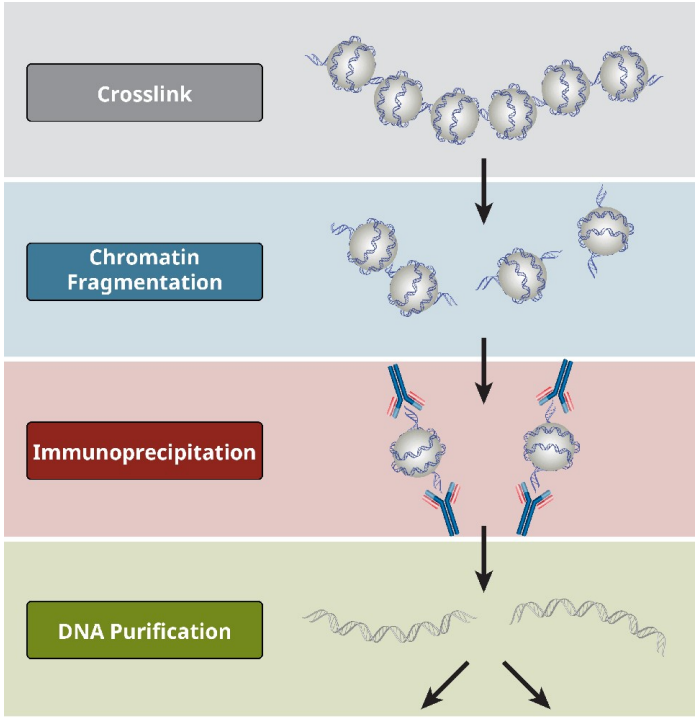
Chromatin Immunoprecipitation (Steps 3&4)

3. Immunoprecipitation
   - Use an antibody to select protein of interest



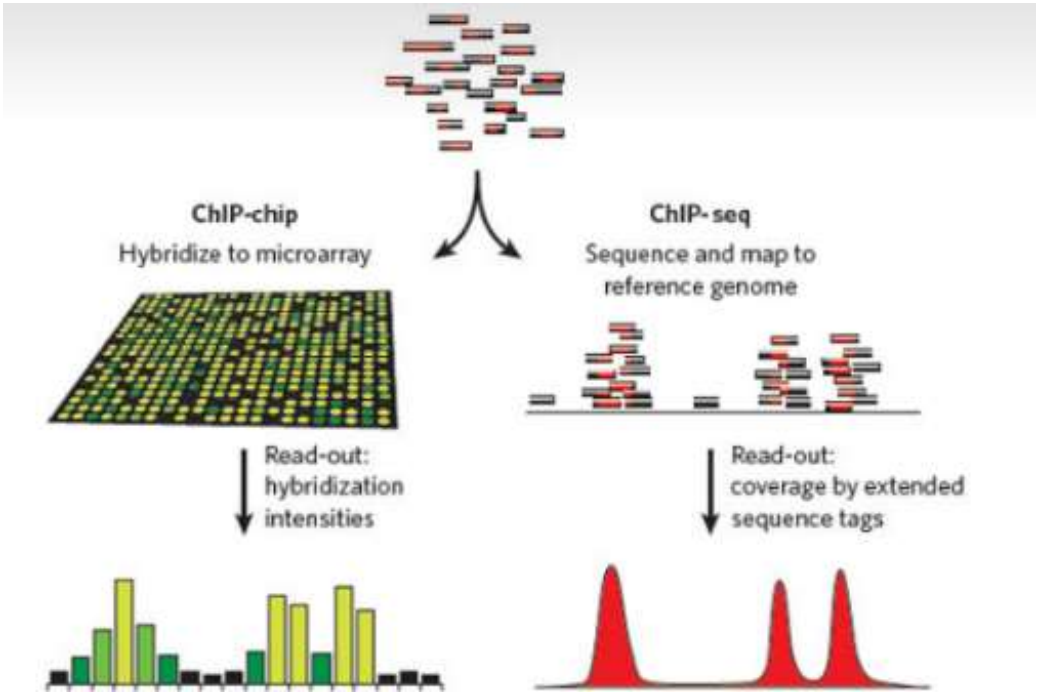
Source: US Biology Life Sciences

4. Purify DNA
   - Cross-link reversal
   - Purify

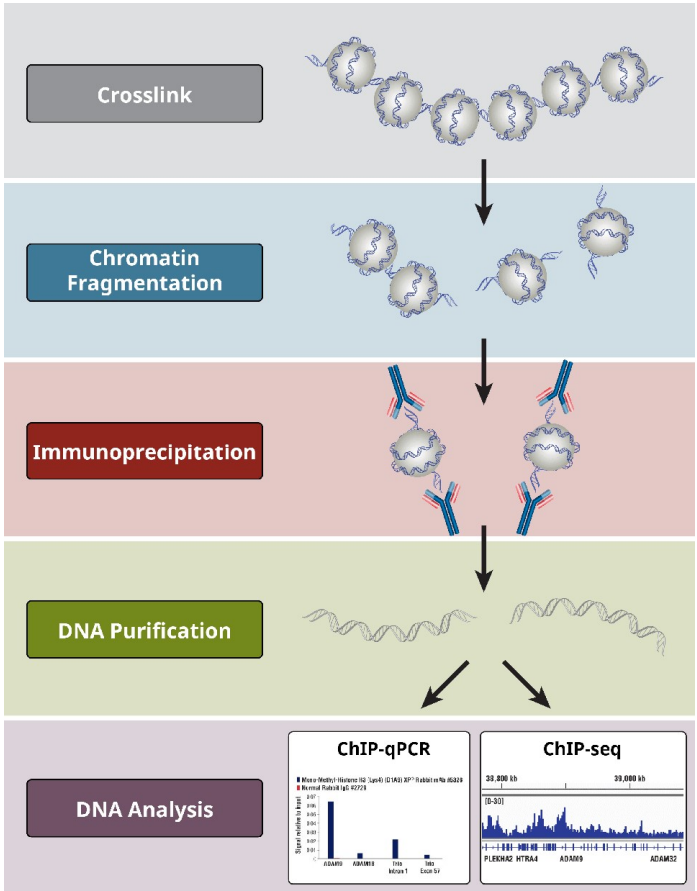


Source: Cell Signaling Technologies

ChIP-Paired With Some DNA Technology



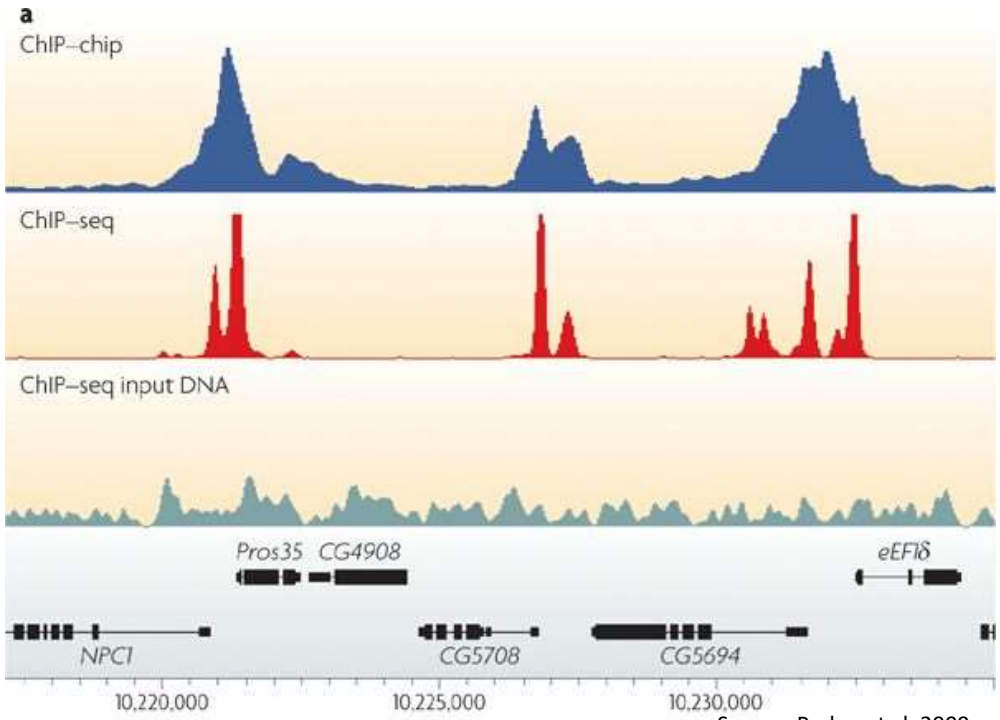
Source: Katerina Kechris



Source: Cell Signaling Technologies

Negative Controls

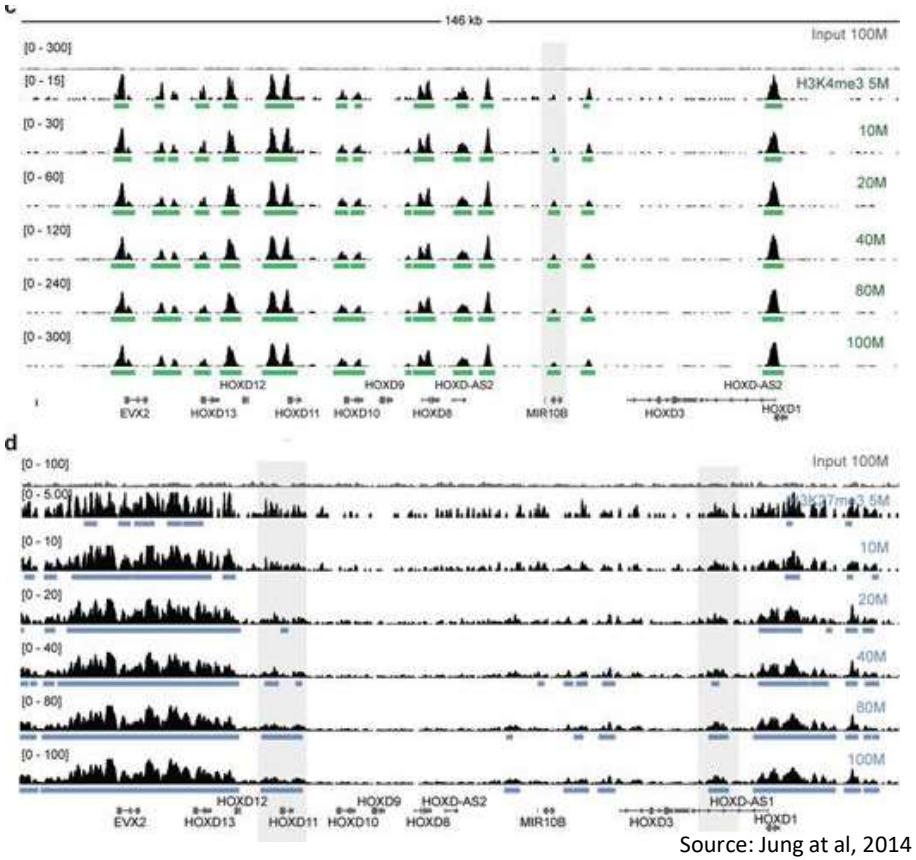
- Sensitivity and specificity of antibody
- Found large artifacts
  - Find peaks in control
- Biases:
  - Shearing of DNA
  - GC content
  - Regions of open chromatin
- Compare your true ChIP-Seq to a background
- Control sample “input” or “IgG”
  - Sonicated chromatin without immunoprecipitation
  - IgG: “unspecific” IP



Source: Parker at al, 2009

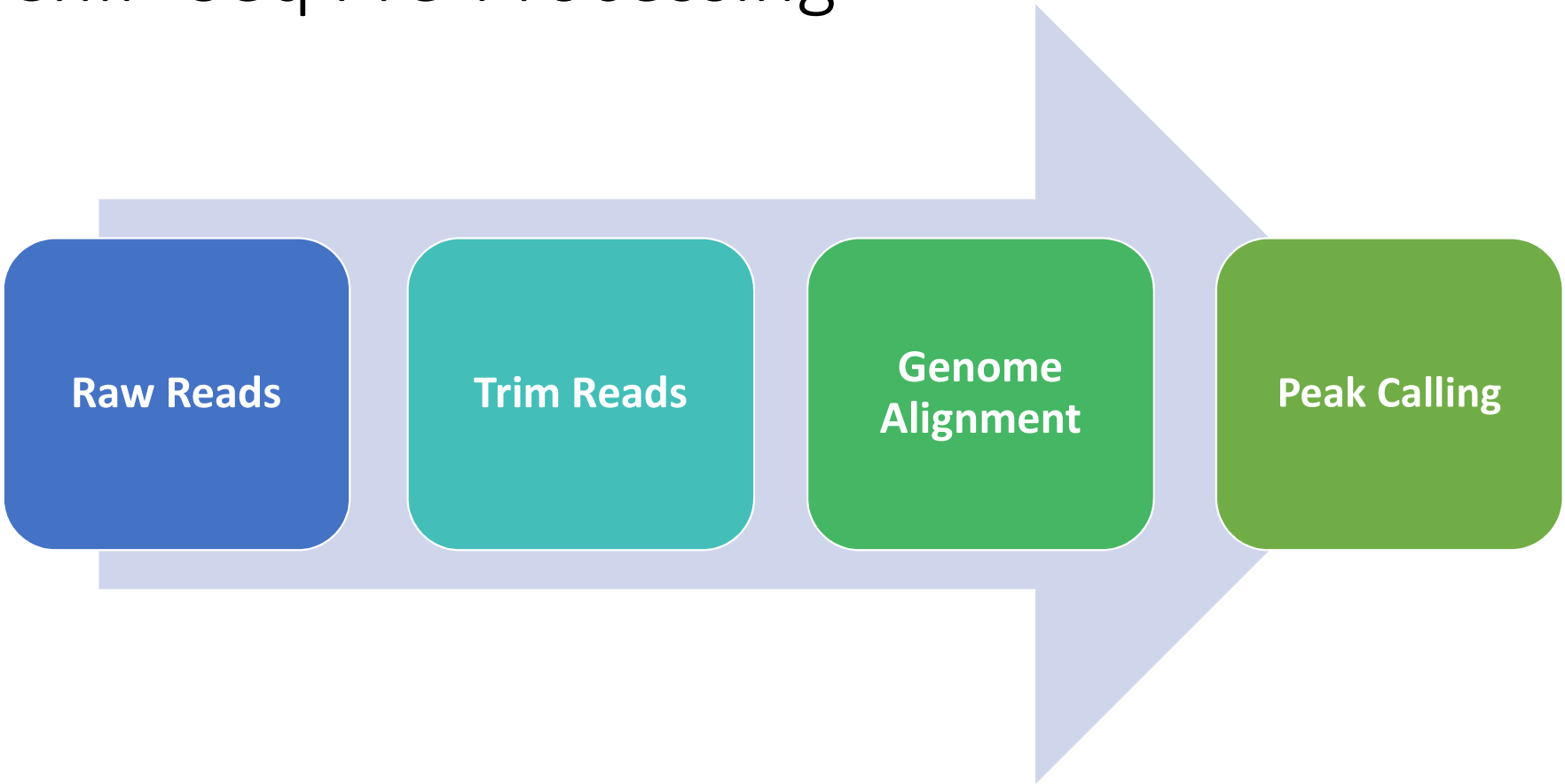
Sequencing & Experimental Design

- Platform
  - Illumina
- Fragment Length
  - 25-35 bp common
- Depth
  - 40-50 Million
- R/CSSP
  - Power analysis for sequencing depth in ChIP-Seq analysis
- Use biological replicates
  - N=3 for cell lines
  - Usually use same control

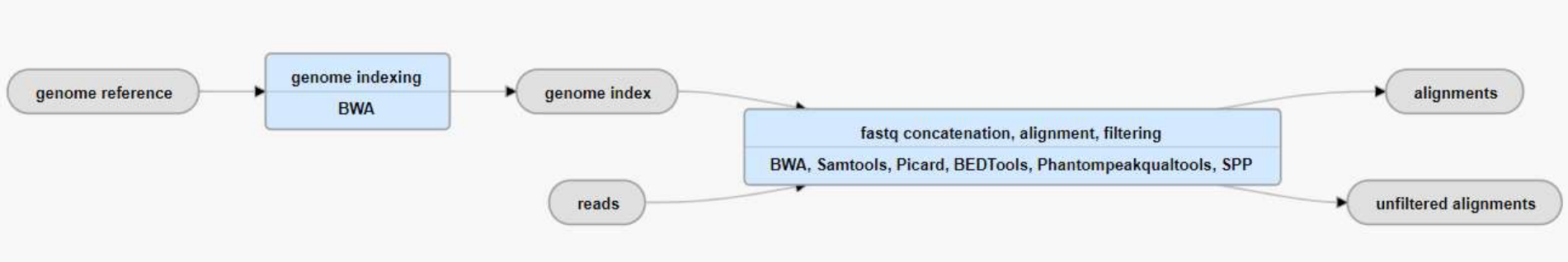


Source: Jung at al, 2014

ChIP-Seq Pre-Processing



ENCODE Pipeline

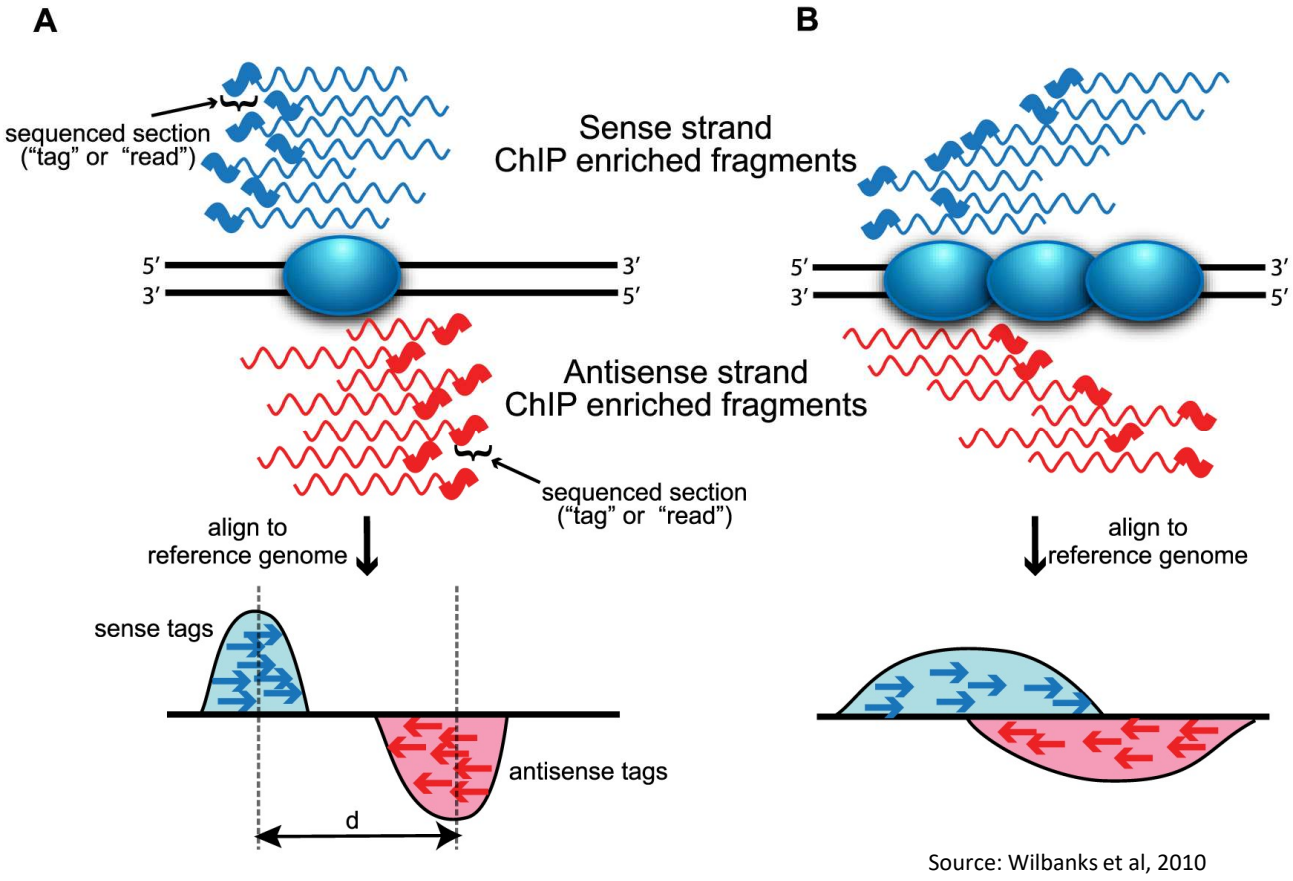


- Raw reads in FASTQ format
- ENCODE recommends using BWA
  - Unspliced aligner (no gaps allowed)
  - Okay because we are dealing with DNA
- Take the aligned bam file to a peak calling algorithm

- QC
  - Uniquely mapped reads, at most 2 mismatches
  - >%50 total reads uniquely mappable
  - >50% reads non-redundant
    - Non-Redundant Fraction (NRF)
    - Need NRF > 0.8 for 10M reads
  - Wide distribution across genome, low GC bias, etc.

Peak Calling

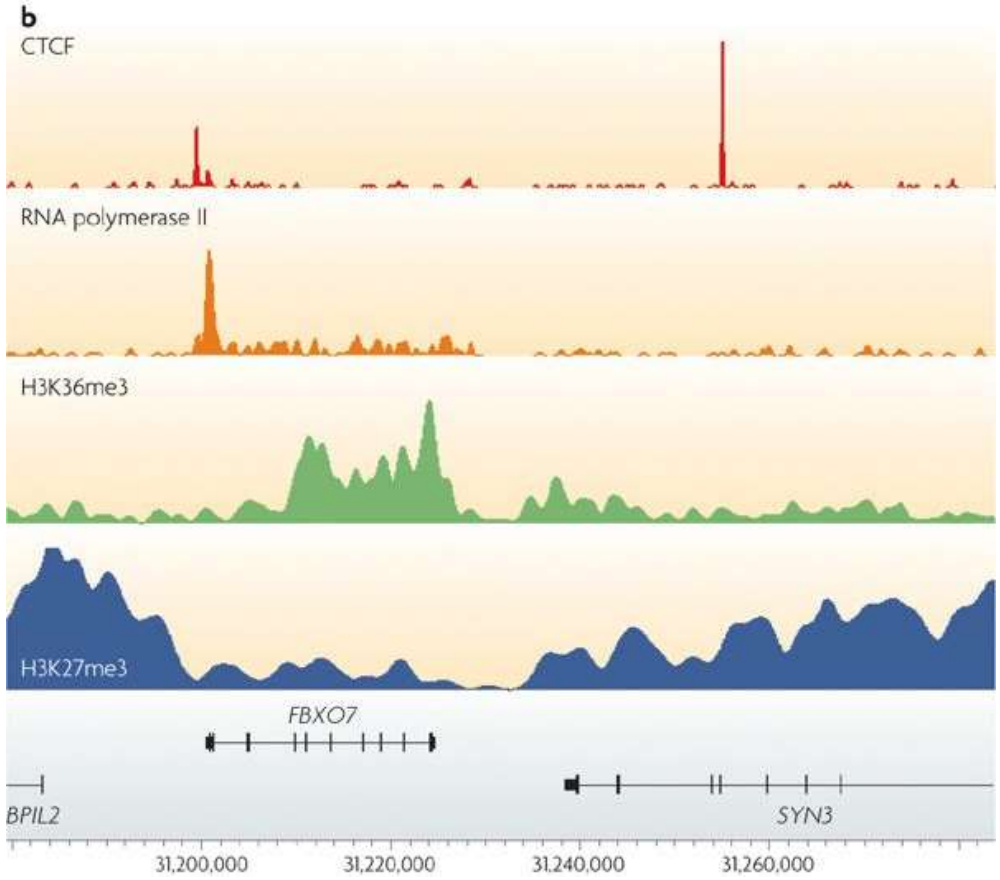
- What area of the genome do we say is interacting/binding with protein?
- Since we have DNA, we have 2 strands
  - Sense
  - Antisense
- A) TF B) Histone



Source: Wilbanks et al, 2010

Histone vs TF Peaks

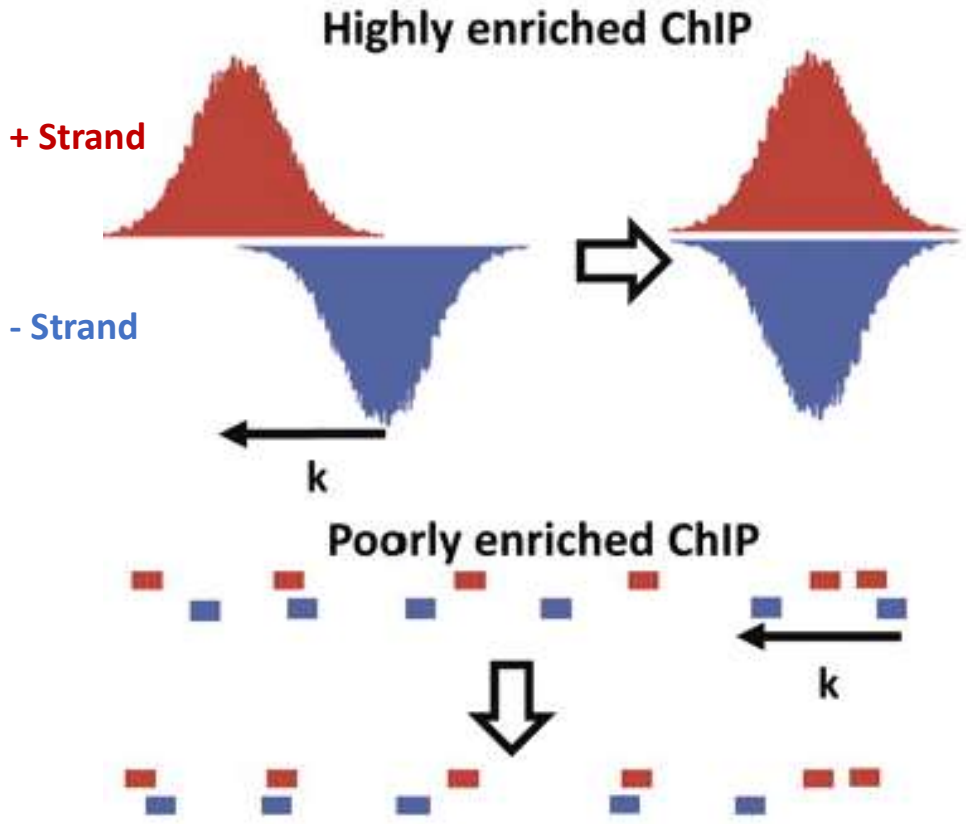
- Transcription factor peaks are sharper
- Transcription factor DNA binding sites are typically 10 nt long
- Histone peaks typically ~4x times length as TF peaks
- Use different peak calling algorithms based on type of ChIP you used



Source: Parker at al, 2009

Read Shifting

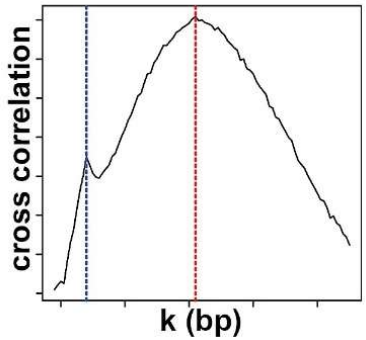
- Offset in forward or reverse strand reads
- Majority ChIP-Seq single-end sequencing
- Reads align to either the sense/antisense strands and the 3’ or 5’ extremes of the DNA fragments pulled down.
- The reads are shifted and the data from both strands combined to determine the most likely bases involved in protein binding.
- How big a "shift" is determined by the fragment size generated in the ChIP-seq library preparation
  - Estimate from sequence data
  - Determine empirically



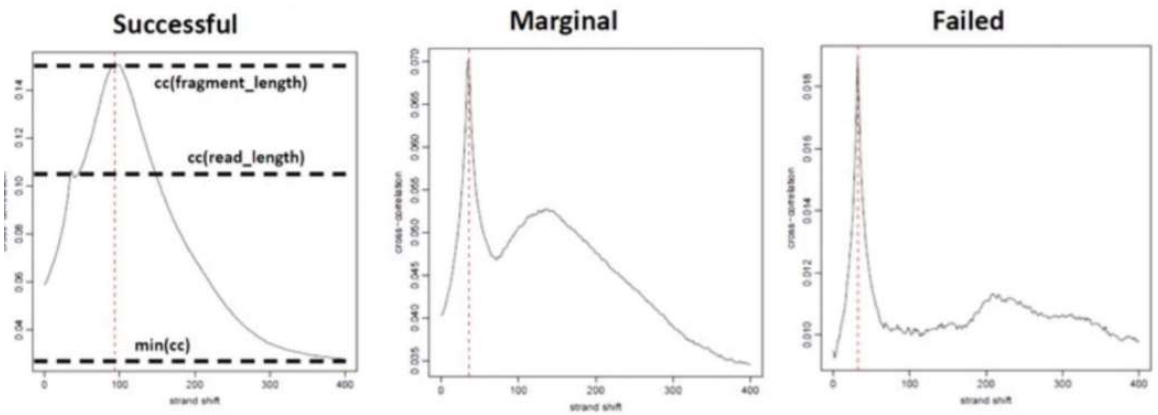
Source: Indiana University

Cross-Correlation

- Pearson correlation between positive and negative strand profiles at different strand shift distances, k.
- Cross-correlation peaks at 2 places:
  - Read length (blue)
  - Average fragment length (red)



Source: Bailey et al, 2013



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

Bad data with NSC values < 1.05 and RSC values < 0.8

Source: Cornell University

Normalization

- Want to compare ChIP to control/input
- Total read counts between ChIP and control/input not the same
- Unfortunately the control/input does not have a uniform distribution of reads across genome
  - GC content, CNV, mappability
- Issue is ChIP really consists of 2 distributions (IP fragments + background) where the control/input just consists of 1 distribution (background)
- Estimate background reads ratio in ChIP reads
  - Scale by ChIP/control normalization factor
  - N_1 and N_2 total reads for ChIP and control
  - $\Pi_0 N_1$ background and $(1 - \Pi_0)\ N_1$ enriched signal reads
  - r is the normalization factor

$$r = \frac{\Pi_0 * N_1}{N_2}$$

Peak Calling Programs

Within R:
BayesPeak, PICS, MOSAiCS, iSeq, ChIPseqR, CSAR, ChIP-Seq, SPP, NarrowPeaks

Command Line Tools:
CisGenome, **E-RANGE**, FindPeaks, F-Seq, GLITR, **MACS,** PeakSeq, QuEST, SICER, SiSSRs, spp, Useq, MUSIC, BCP

The options are endless!

Program	Reference	Version	Graphical user interface?	Window-based scan	Tag clustering	Gaussian kernel density estimator	Strand-specific scoring	Peak height or fold enrichment (FE)	Background subtraction	Compensates for genomic duplications or deletions	False Discovery Rate	Compare to normalized control data (FE)	Compare to statistical model fitted with control data	Statistical model or test
CisGenome	28	1.1	X*	X				X	X		X		X	conditional binomial model
Minimal ChipSeq Peak Finder	16	2.0.1			X			X				X		
E-RANGE	27	3.1			X			X				X	X	chromsome scale Poisson dist.
MACS	13	1.3.5		X				X			X		X	local Poisson dist.
QuEST	14	2.3				X		X			X**		X	chromsome scale Poisson dist.
HPeak	29	1.1		X				X					X	Hidden Markov Model
Sole-Search	23	1	X	X				X		X			X	One sample t-test
PeakSeq	21	1.01			X			X					X	conditional binomial model
SISSRS	32	1.4		X			X					X		
spp package (wtd & mtc)	31	1.7		X			X		X	X'	X			
				Generating density profiles			Peak assignment		Adjustments w. control data		Significance relative to control data			

X* = Windows-only GUI or cross-platform command line interface

X** = optional if sufficient data is available to split control data

X' = method exludes putative duplicated regions, no treatment of deletions

Source: Parker at al, 2009

New Recommendations

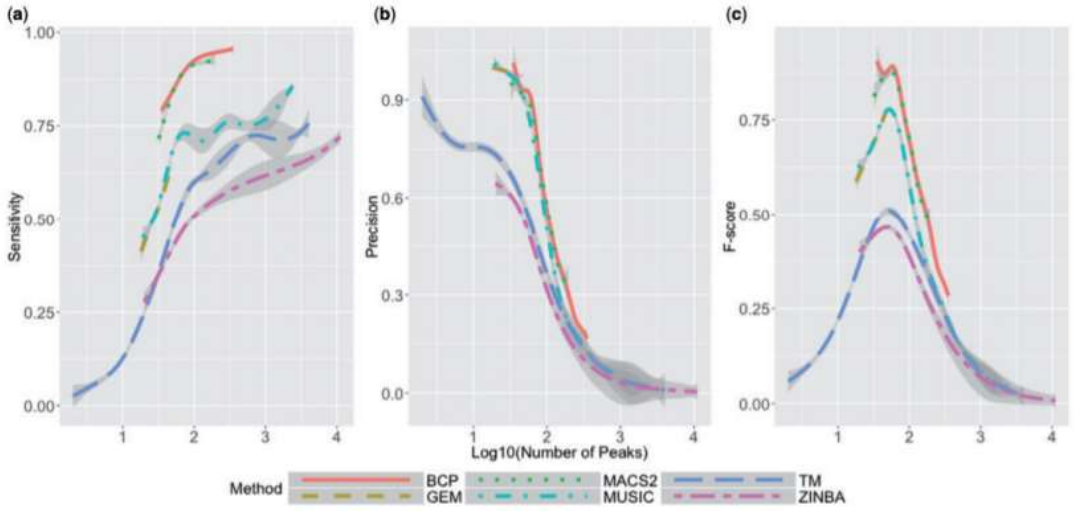
- Poisson test to rank their candidate peaks are more powerful than those that use a Binomial test
- Best for TF:
  - BCP and MACS2
- Best for Histones:
  - BCP and MUSIC

Briefings in Bioinformatics, 18(3), 2017, 441–450

doi: 10.1093/bib/bbw035
Advance Access Publication Date: 11 May 2016
Paper

Features that define the best ChIP-seq peak calling algorithms

Reuben Thomas, Sean Thomas, Alisha K. Holloway and Katherine S. Pollard



MACS/MACS2

1. Removes redundant reads
   - Detects read length automatically
   - Filters duplicates
   - Calculates max # duplicate reads in single positions warranted by sequencing depth, removes excess of this number
2. Accounts for read-shifting for the offset in forward or reverse strand reads
   - Models distance between paired forward and reverse strand peaks
   - Slides window across genome to find enriched regions (M-fold > background)
   - Size bandwidth is 2x bandwidth parameter (can opt for a broad option for histones)
   - Expected background # reads*length/mappable genome size
3. Peak Detection Phase
   - Extends reads in 3' direction to the fragment length from modeling
   - Scales samples linearly to same read number of control/input
   - Scans genome again window size 2x fragment length
   - P-value from dynamic Poisson distribution capture local biases and read background levels
   - Benjamini-Hochberg FDR correction

MACS2 Code

Filter duplicates for each bam file

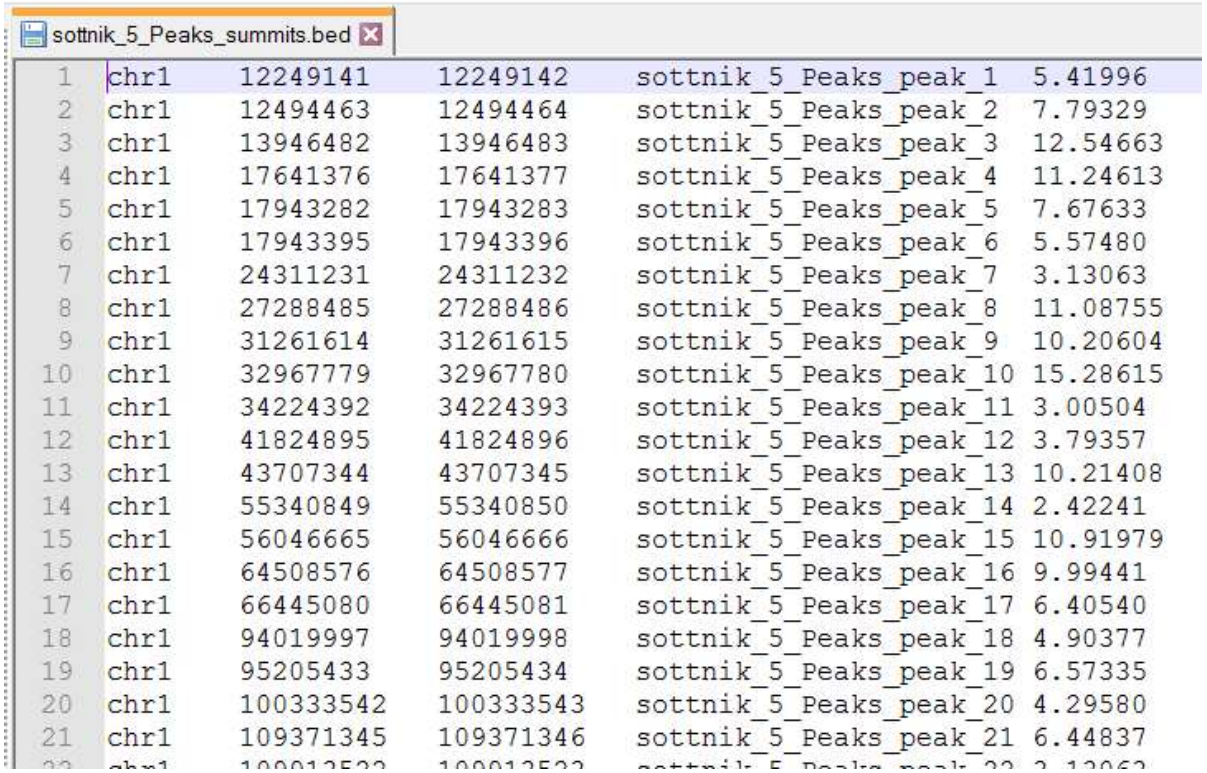
-i input file
-f format of input file
-g genome you aligned to
--keep-dup number of duplicates to keep
-o output file

```
# Filter duplicates
# Write down the count of reads for each sample after duplicate removal

macs2 filterdup \
-i "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/tp53_r2.fastq_trimmed.fastq_sorted.bam" \
-f BAM -g hs --keep-dup=1  --verbose=3 -o "tp53_r2.fastq_trimmed.fastq_sorted_filterdup.bed"

macs2 filterdup \
-i "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/TAp73beta_r2.fastq_trimmed.fastq_sorted.bam" \
-f BAM -g hs --keep-dup=1  --verbose=3 -o "TAp73beta_r2.fastq_trimmed.fastq_sorted_filterdup.bed"

macs2 filterdup \
-i "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/input.fastq_trimmed.fastq_sorted.bam" \
-f BAM -g hs --keep-dup=1  --verbose=3 -o "input.fastq_trimmed.fastq_sorted_filterdup.bed"
```

BED File Format

- Tab delimited file
- No Column Headers
- First 3 columns required

Column 1	Chromosome
Column 2	Start (bp)
Column 3	End (bp)
Column 4	Name
Column 5	Score
Column 6	Strand



MACS2 Code

- Predict Fragment Length

-i	input file
-g	genome you aligned to
-m	M-FOLD Range to look at (default is 5 – 50)
-bw	bandwidth of region used to compute fragment size (default 300)

```
# predict fragment length
# write down the fragment length for each sample

macs2 predictd -i tp53_r2.fastq_trimmed.fastq_sorted_filterdup.bed -g hs -m 5 20
macs2 predictd -i TAp73beta_r2.fastq_trimmed.fastq_sorted_filterdup.bed -g hs -m 5 20
macs2 predictd -i input.fastq_trimmed.fastq_sorted_filterdup.bed -g hs -m 5 20
```

MACS2 Code

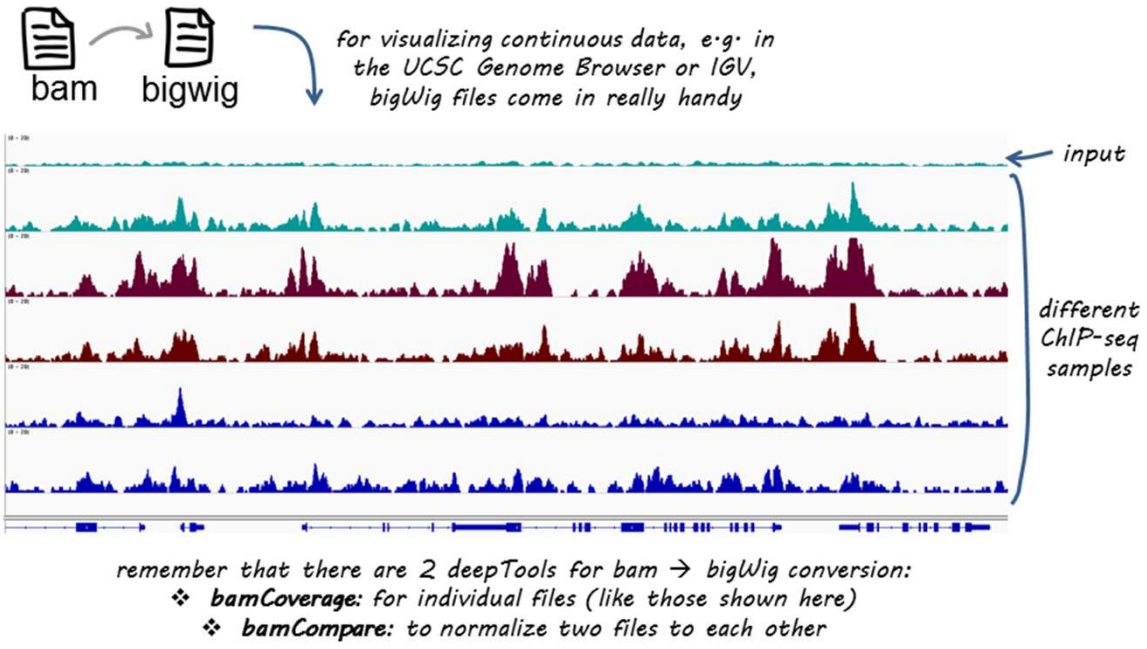
-t	ChIP file
-c	control/input file
-n	names to generate output
--broad	look for broad peaks (histones)
--bgd	generate bedgraph files

```
# MACS2 callpeak options
macs2 callpeak -h

# -t sample -c control -g effective genome size needs to be empirically computed using
# a hg38.fa genome file for
# hg38 but for this practical use 'hs' which is = 2.6e9, the value for hg19
# -f filetype --bdg generate bedgraph
```

```
macs2 callpeak \
-t "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/tp53_r2.fastq_trimmed.fastq_sorted.bam" \
-c  "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/input.fastq_trimmed.fastq_sorted.bam" \
-g hs -n tp53_r2.fastq_trimmed.fastq_sorted_standard -f BAM --keep-dup auto --bdg

macs2 callpeak \
-t "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/TAp73beta_r2.fastq_trimmed.fastq_sorted.bam" \
-c "~/Course_Materials/ChIPSeq/Preprocessed/Alignment_BWA/input.fastq_trimmed.fastq_sorted.bam" \
-g hs -n TAp73beta_r2.fastq_trimmed.fastq_sorted_standard -f BAM --keep-dup auto --bdg
```

Bedgraph & BigWig Files

- For visualization specifically in the genome browser



deeptools
samtools

Provide good options for going back and forth between file formats

MAC2 Output

	A
1	# This file is generated by MACS version 2.1.0.20150731
2	# Command line: callpeak -t sottnik_5.markdup.Sample.bed -c controlSample.bed -f BED -g hs -n sottnik_5_Peaks --outdir /data/home/vanderll/Sottnik/data_processed/v1.alreadyAligned/sottnik_5_peaks -B --SPMR
3	# ARGUMENTS LIST:
4	# name = sottnik_5_Peaks
5	# format = BED
6	# ChIP-seq file = ['sottnik_5.markdup.Sample.bed']
7	# control file = ['controlSample.bed']
8	# effective genome size = 2.70e+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	# MACS will save fragment pileup signal per million reads
16	
17	# tag size is determined as 49 bps
18	# total tags in treatment: 24339985
19	# tags after filtering in treatment: 24339985
20	# maximum duplicate tags at the same position in treatment = 1
21	# Redundant rate in treatment: 0.00
22	# total tags in control: 24339980
23	# tags after filtering in control: 24339980
24	# maximum duplicate tags at the same position in control = 1
25	# Redundant rate in control: 0.00
26	# d = 49
27	# alternative fragment length(s) may be 49 bps

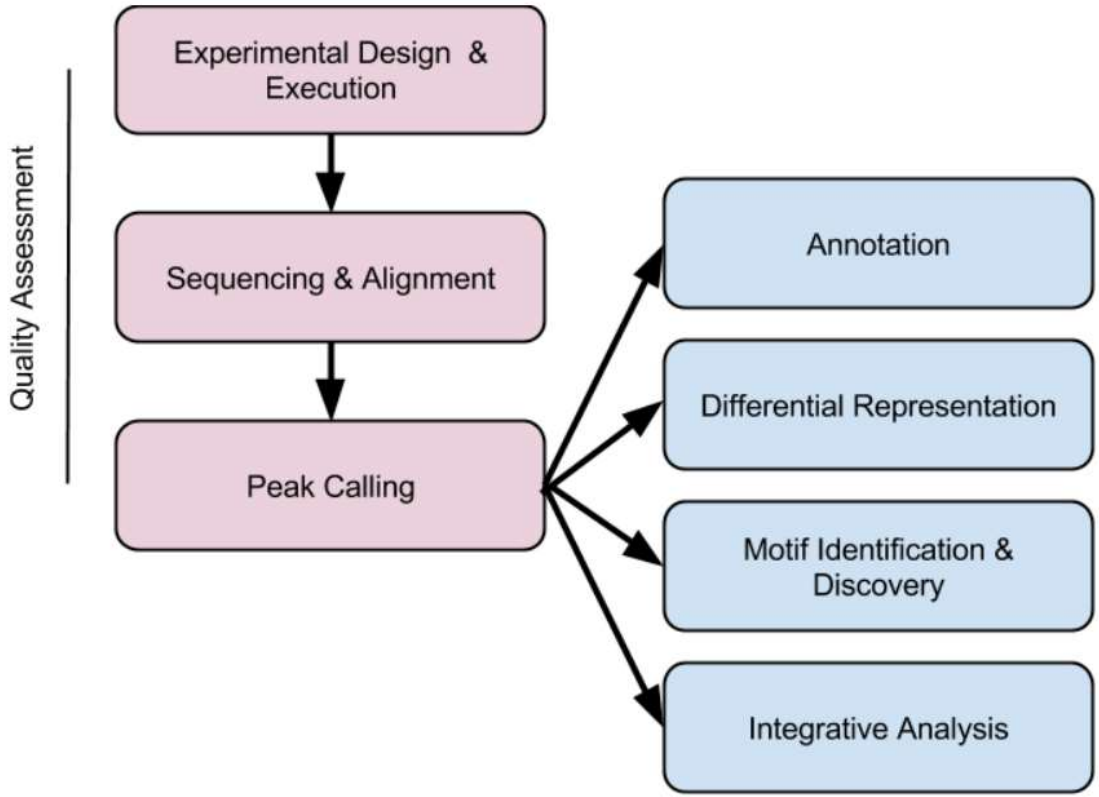
MACS2 Output

-log10(p-value) -log10(q-value)

Absolute summit Fold Enrichment

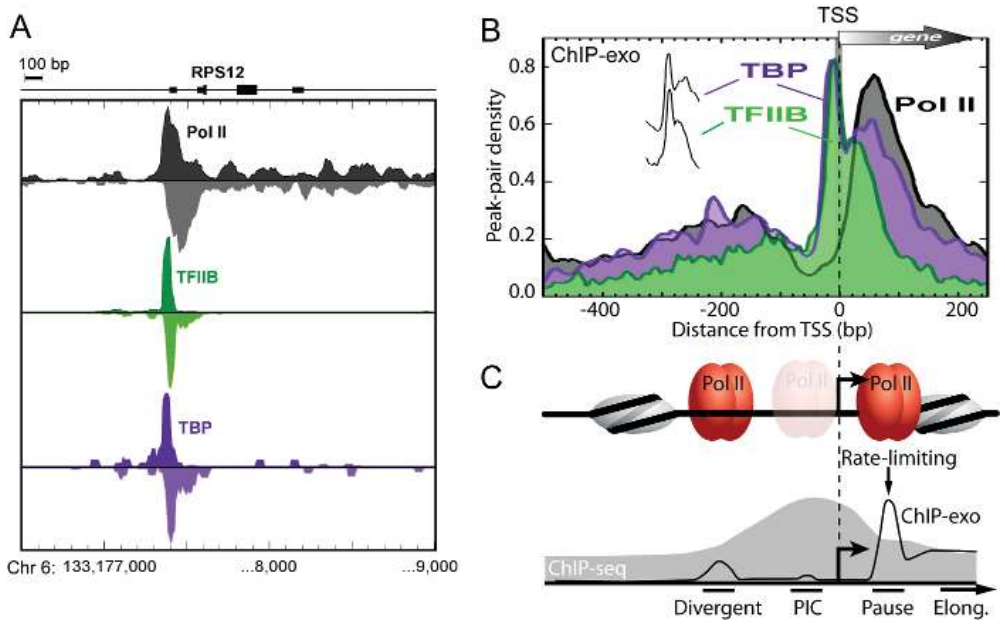
27	# alternative fragment length(s) may be 49 bps									
28	chr	start	end	length	abs_summ	pileup	-LOG10(pv	fold_enric	-LOG10(qv	name
29	chr1	12249114	12249173	60	12249142	10	10.10805	7.53115	5.41996	sottnik_5_Peaks_peak_1
30	chr1	12494400	12494509	110	12494464	15	12.64637	8.08081	7.79329	sottnik_5_Peaks_peak_2
31	chr1	13946446	13946680	235	13946483	16	17.83532	11.04613	12.54663	sottnik_5_Peaks_peak_3
32	chr1	17641249	17641549	301	17641377	27	16.40858	7	11.24613	sottnik_5_Peaks_peak_4
33	chr1	17943202	17943304	103	17943283	15	12.52288	8	7.67633	sottnik_5_Peaks_peak_5
34	chr1	17943361	17943415	55	17943396	13	10.30259	7.07071	5.5748	sottnik_5_Peaks_peak_6
35	chr1	24311219	24311307	89	24311232	10	7.68112	6.00109	3.13063	sottnik_5_Peaks_peak_7
36	chr1	27288454	27288541	88	27288486	17	16.24134	9.81997	11.08755	sottnik_5_Peaks_peak_8
37	chr1	31261495	31261660	166	31261615	22	15.25735	7.66667	10.20604	sottnik_5_Peaks_peak_9
38	chr1	32967664	32967826	163	32967780	18	20.85935	12.34568	15.28615	sottnik_5_Peaks_peak_10
39	chr1	34224371	34224600	230	34224393	14	7.53305	5	3.00504	sottnik_5_Peaks_peak_11
40	chr1	41824872	41824930	59	41824896	13	8.38157	5.78273	3.79357	sottnik_5_Peaks_peak_12
41	chr1	43707163	43707459	297	43707345	15	15.26552	9.74481	10.21408	sottnik_5_Peaks_peak_13

Analyses with Peaks



Peak Annotation

- Is my peak in an gene?
- What about promoter region?
- Enhancer region?
- Could just look at the nearest gene and calculate distance from gene
- R/ChIPseeker helps annotate peaks



Source: Perreault et al, 2016

ChIPseeker Code

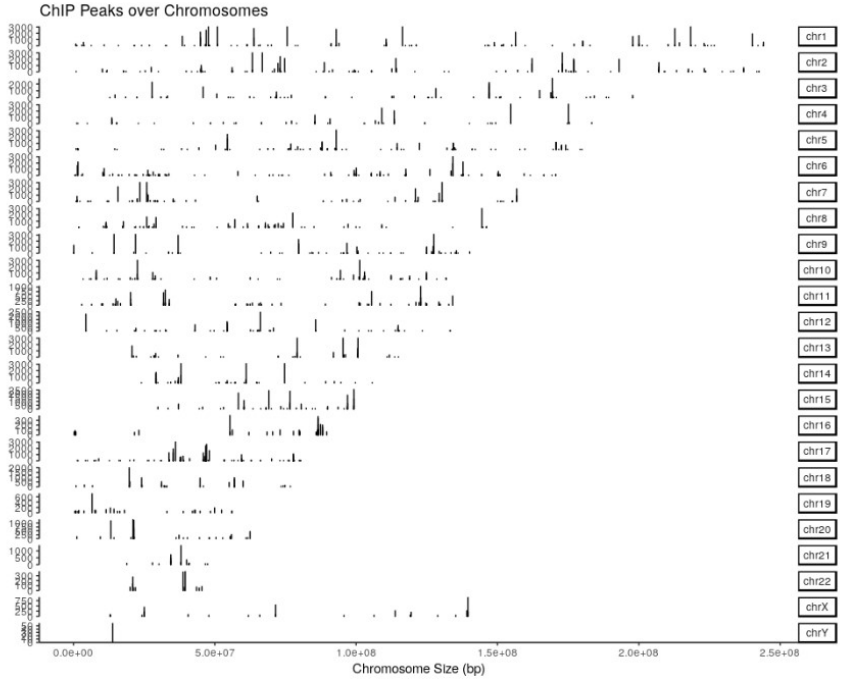
```
## loading packages
library(ChIPseeker)
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
library(clusterProfiler)

##read in peak calls (bed file)
peak <- readPeakFile("/path/macs2_peaks.bed")
```

peak is a GRanges object,
which is a type of S4 object

```
## GRanges object with 1331 ranges and 2 metadata columns:
##          seqnames              ranges strand |             V4        V5
##             <Rle>           <IRanges>  <Rle> |       <factor> <numeric>
##      [1]     chr1       815093-817883      * |    MACS_peak_1    295.76
##      [2]     chr1     1243288-1244338      * |    MACS_peak_2     63.19
##      [3]     chr1     2979977-2981228      * |    MACS_peak_3    100.16
##      [4]     chr1     3566182-3567876      * |    MACS_peak_4    558.89
##      [5]     chr1     3816546-3818111      * |    MACS_peak_5     57.57
##      ...      ...                 ...    ... .            ...       ...
##   [1327]     chrX 135244783-135245821      * | MACS_peak_1327     55.54
##   [1328]     chrX 139171964-139173506      * | MACS_peak_1328    270.19
##   [1329]     chrX 139583954-139586126      * | MACS_peak_1329    918.73
##   [1330]     chrX 139592002-139593238      * | MACS_peak_1330    210.88
##   [1331]     chrY   13845134-13845777      * | MACS_peak_1331     58.39
##   -------
##   seqinfo: 24 sequences from an unspecified genome; no seqlengths
```

ChIPseeker Code – coverage plot

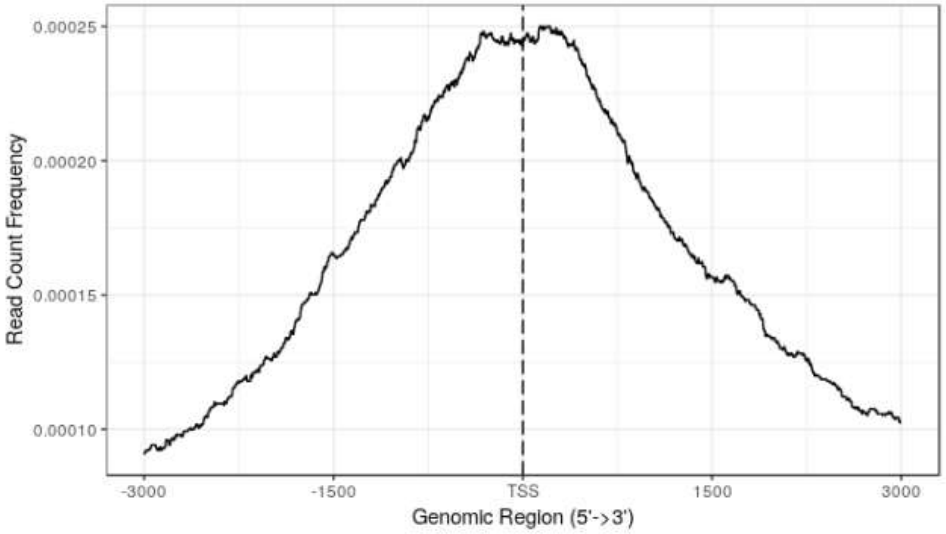
```
covplot(peak, weightCol="V5")
```



ChIPseeker Code – TSS Distance Code

```
#find what you consider promoter regions
promoter <- getPromoters(TxDb=txdb, upstream=3000, downstream=3000)
##find distance peaks are to defined promoter regions
tagMatrix <- getTagMatrix(peak, windows=promoter)
```

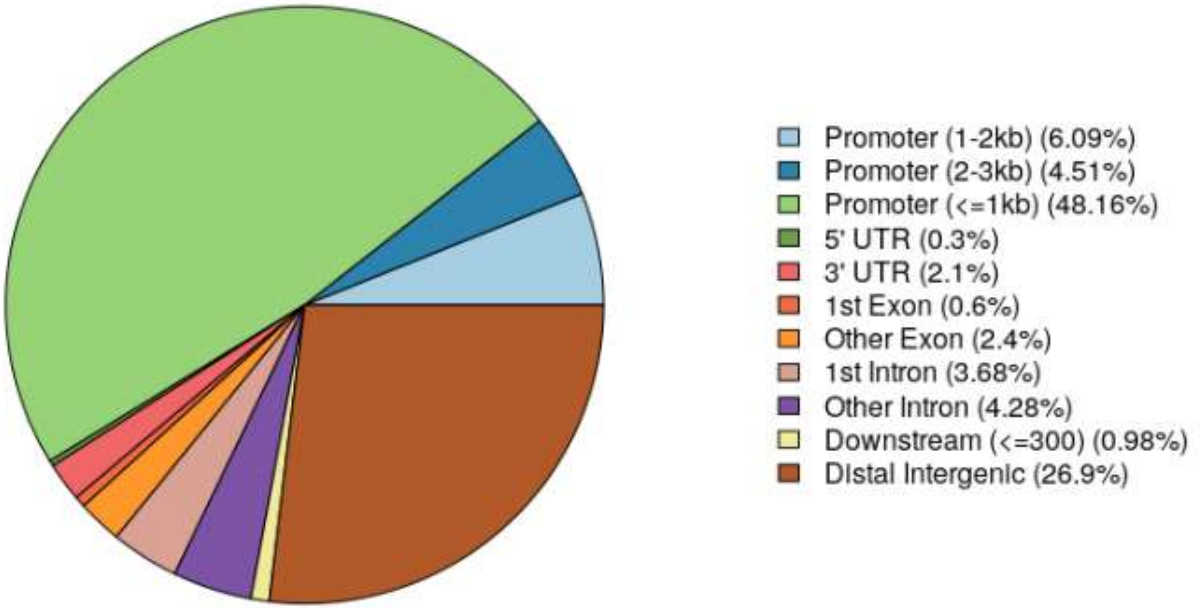
```
plotAvgProf(tagMatrix, xlim=c(-3000, 3000),
            xlab="Genomic Region (5'->3')", ylab = "Read Count Frequency")
```



ChIPseeker Code – Peak Annotation

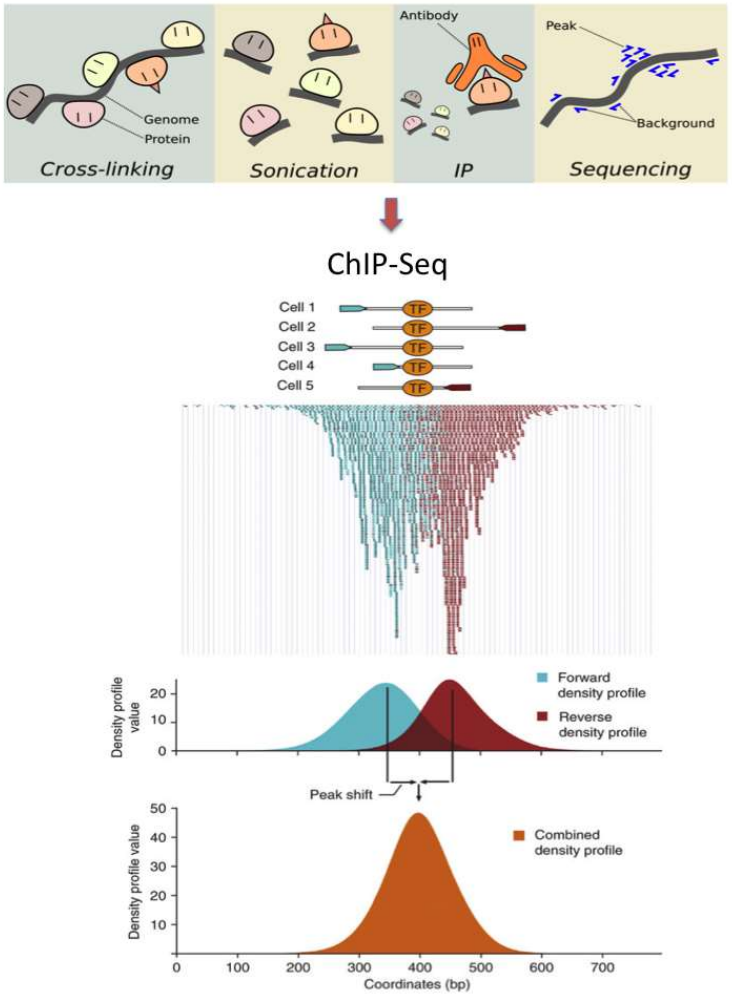
```
##get peak annotation
peakAnno <- annotatePeak("/path/macs2_peaks.bed", tssRegion=c(-3000, 3000),
                         TxDb=txdb, annoDb="org.Hs.eg.db")
```

```
plotAnnoPie(peakAnno)
```



Summary

- Use chromatin immunoprecipitation to extract DNA fragments which bind to protein of interest
- Make peak calls
- Annotate peak calls



References

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References Continued

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- https://bioinformatics-core-shared-training.github.io/cruk-autumn-school-2017/ChIP/Materials/Practicals/Prctical4_PeakCalling_SS.pdf