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Introduction to ChIP Sequencing and QC

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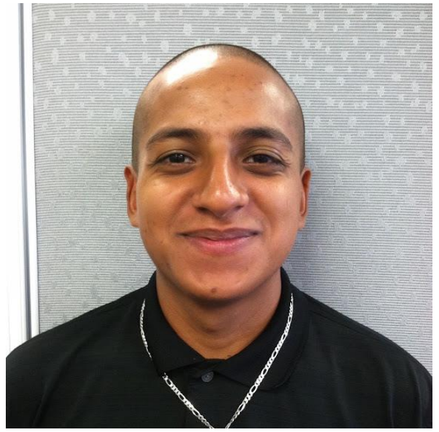
St. Jude Children's Research Hospital
December 11, 2024

The DNB Bioinformatics Core Team



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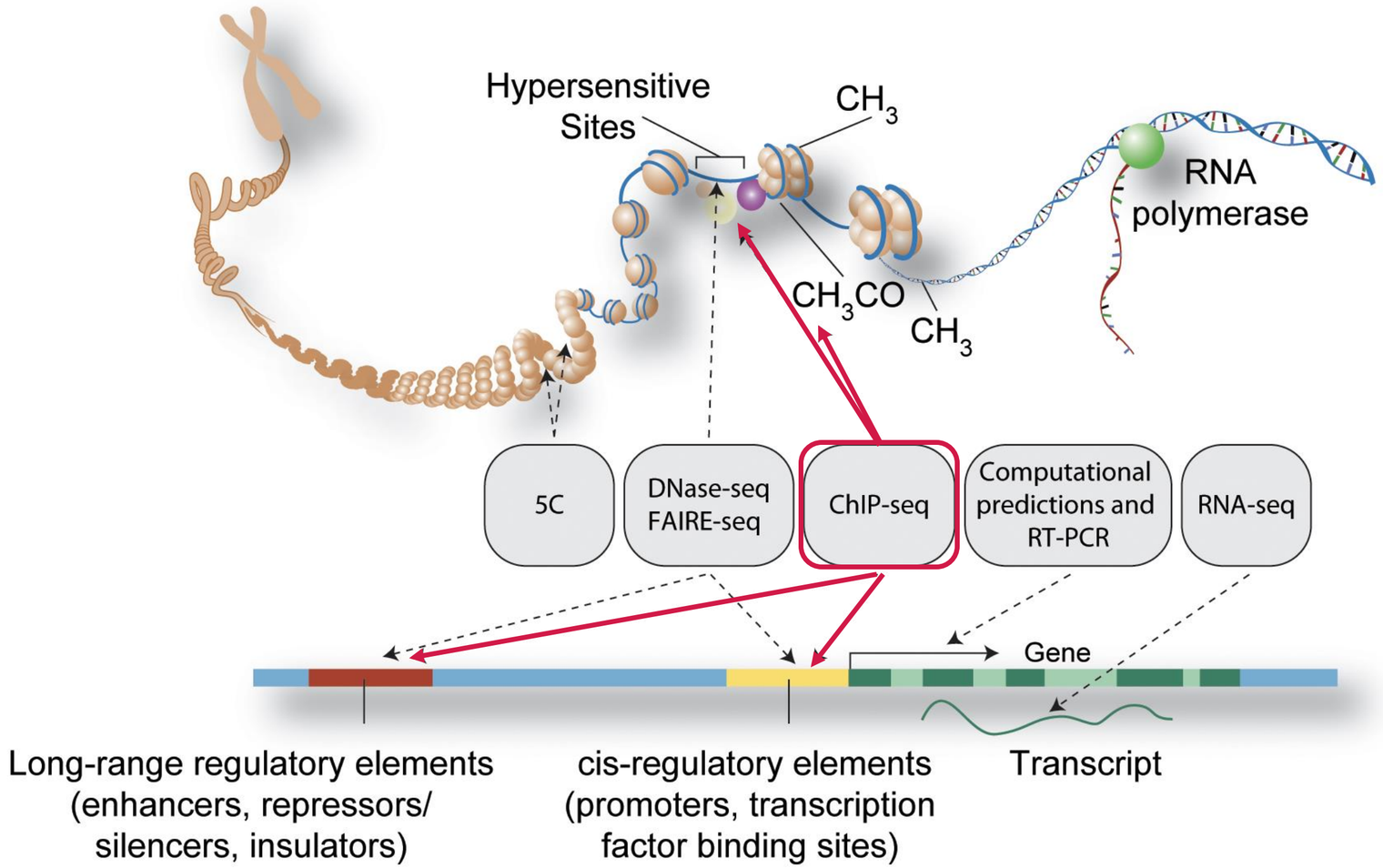
Introduction to ChIP Sequencing and QC Workshop Overview

- ChIP-Seq overview
  - What is ChIP-Seq?
  - How is ChIP-Seq done?
  - What are other methods for profiling regulatory elements?
- Submitting samples for sequencing and analysis
- CAB’s ChIP-Seq pipelines
  - AutoMapper, QC, and Peak Calling
- Example of ChIP-Seq QC and Peak Calling report from CAB

ChIP Sequencing Overview

ChIP sequencing is a useful tool for understanding complex transcriptional regulation



Adapted from The ENCODE Project Consortium (2011). PLOS Biology.

How does ChIP-Seq work?

- Uses a combination of chromatin immunoprecipitation (ChIP) and NGS (seq)
  - Antibody selection for proteins of interest
  - Next generation sequencing
  - Assays protein-DNA binding in vivo, across genome
- Complements gene expression profiling, DNA accessibility methods
- Caveats
  - Qualitative, not quantitative, profiles enrichment
  - Need quite a bit of material for standard ChIP-Seq
  - Heterogeneity can be hard to capture
  - Must have good antibodies for selection step

ChIP-Seq library preparation considerations

Must have sufficient starting material

- At minimum, 10^7 cultured cells recommended for single ChIP experiment

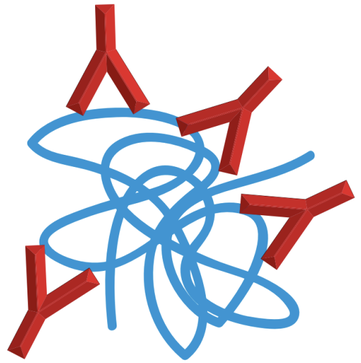
Success dependent on antibody selection

- Should be specific
- Can monoclonal or polyclonal

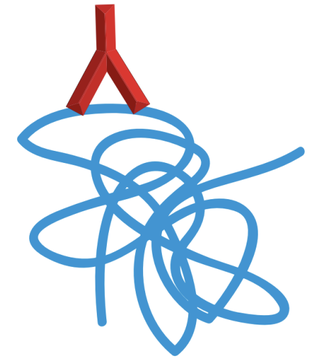
Two general ChIP procedures to choose from:

- Native ChIP (N-ChIP)
- Cross-linking ChIP (X-ChIP)

Polyclonal antibodies



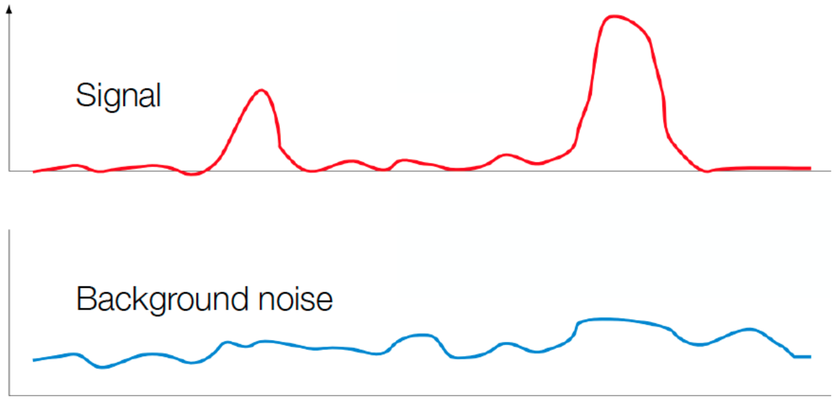
Monoclonal antibodies



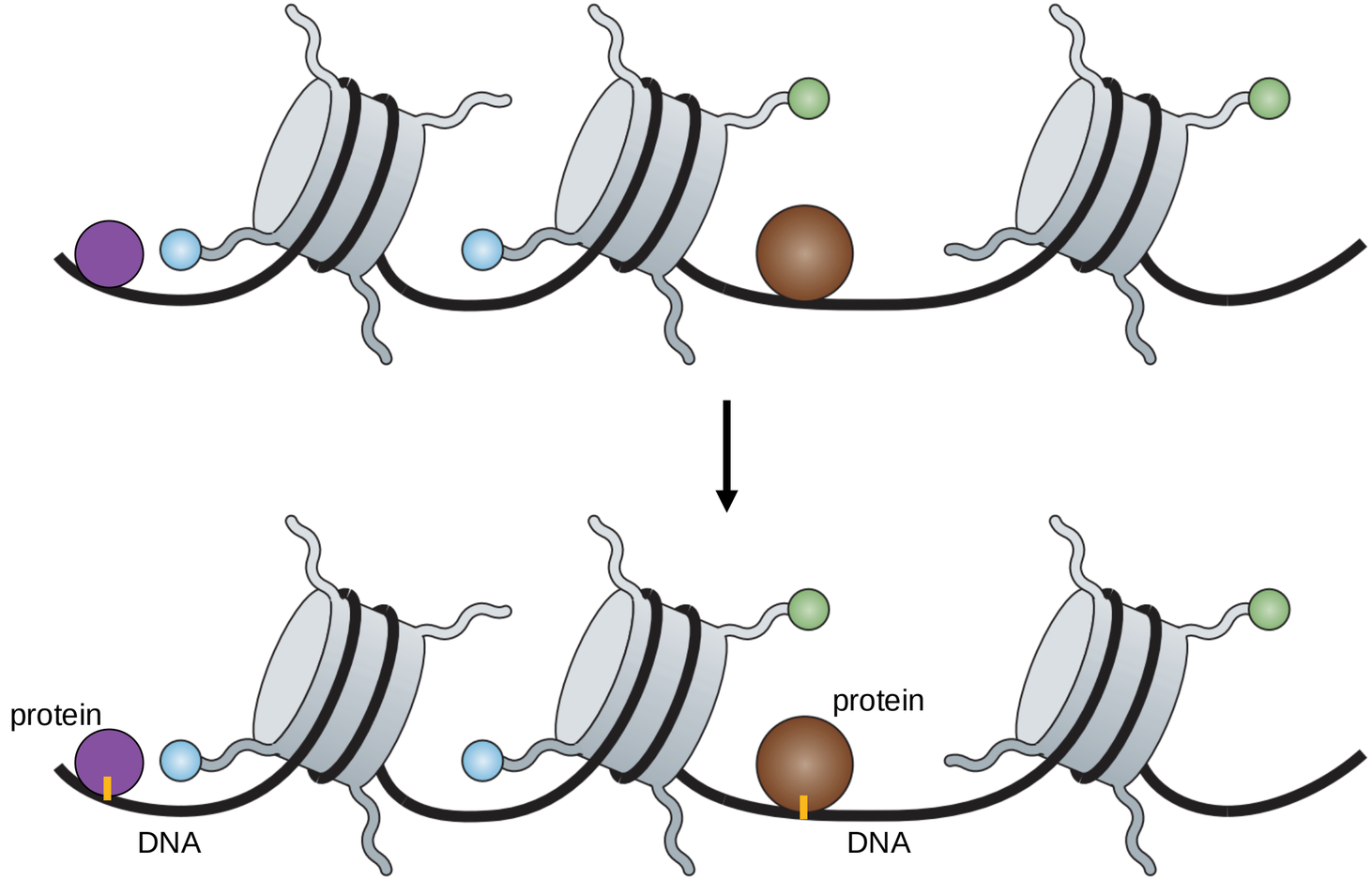
Credit: www.abcam.com/chip

Controls are necessary for ChIP-Seq experiments

- Noise in ChIP experiments not uniform
  - Affected by chromatin confirmation, local biases, mappability
- Need to model background noise to distinguish true peaks
- Input controls are necessary to estimate noise
  - Cross-linked, fragmented DNA without antibody enrichment
  - Recommended one for every immunoprecipitation done
  - If constraints, one per sample group can be sufficient
- Isotype (IgG) controls can also be used
  - Immunoprecipitation with an isotype-matched control
  - Similar to experimental antibody, but non-specific binding
- Can also use positive and negative controls, qPCR to check success
  - Positive: check signal in with expected protein binding
  - Negative: check lack of signal in non-enriched region

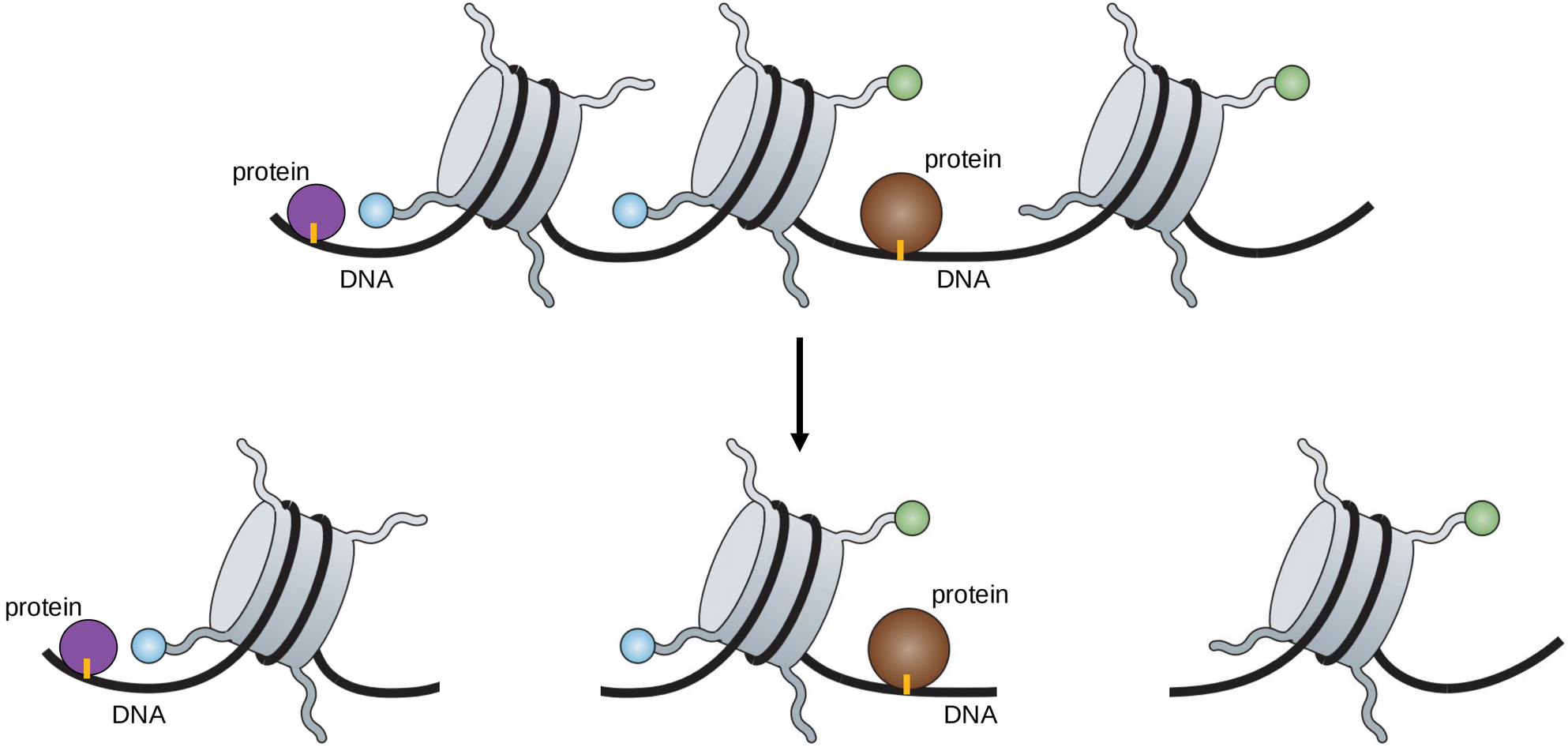


Cross-linking proteins and DNA is (often) the first step for ChIP-Seq library prepartion



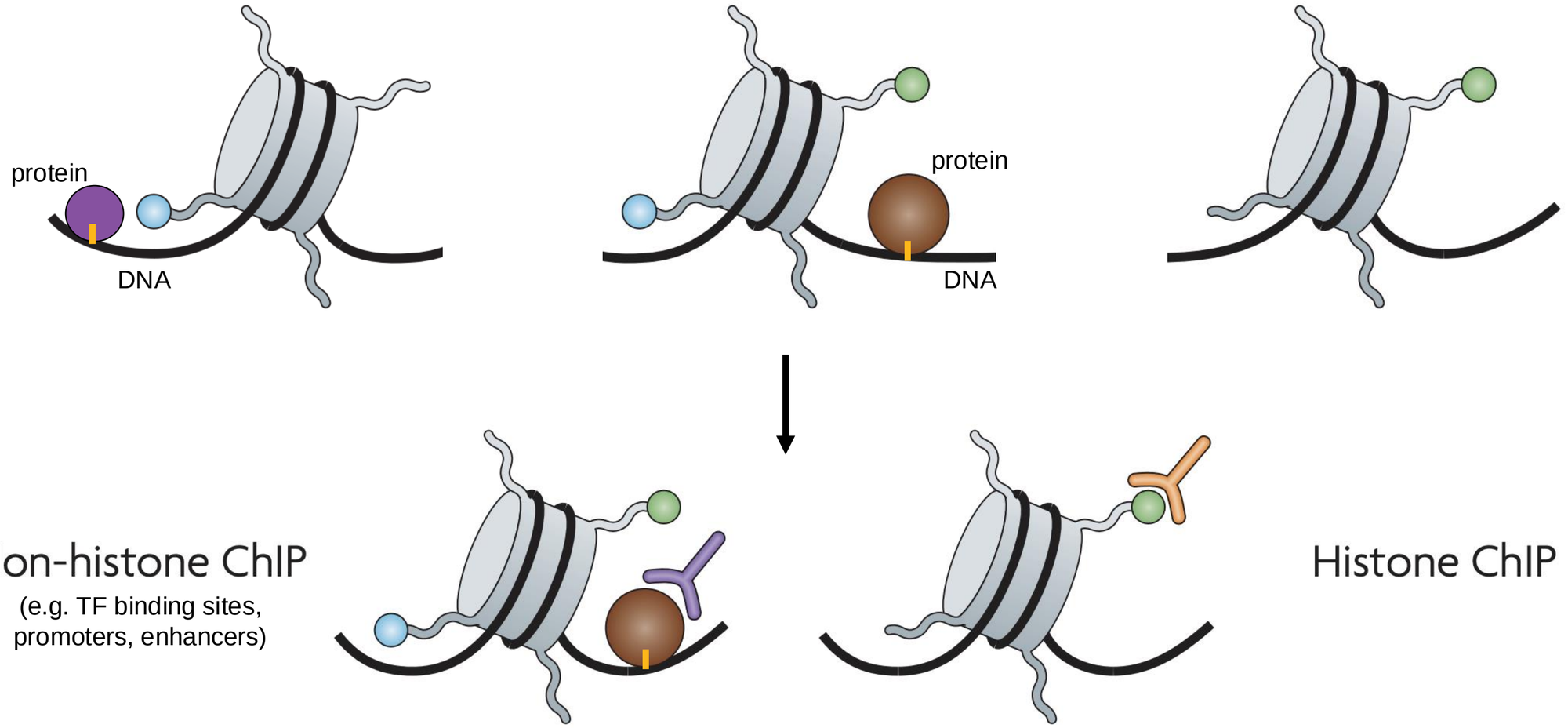
Adapted from Park (2009). Nature Reviews Genetics.

Next step for ChIP-Seq preparation is fragmenting DNA



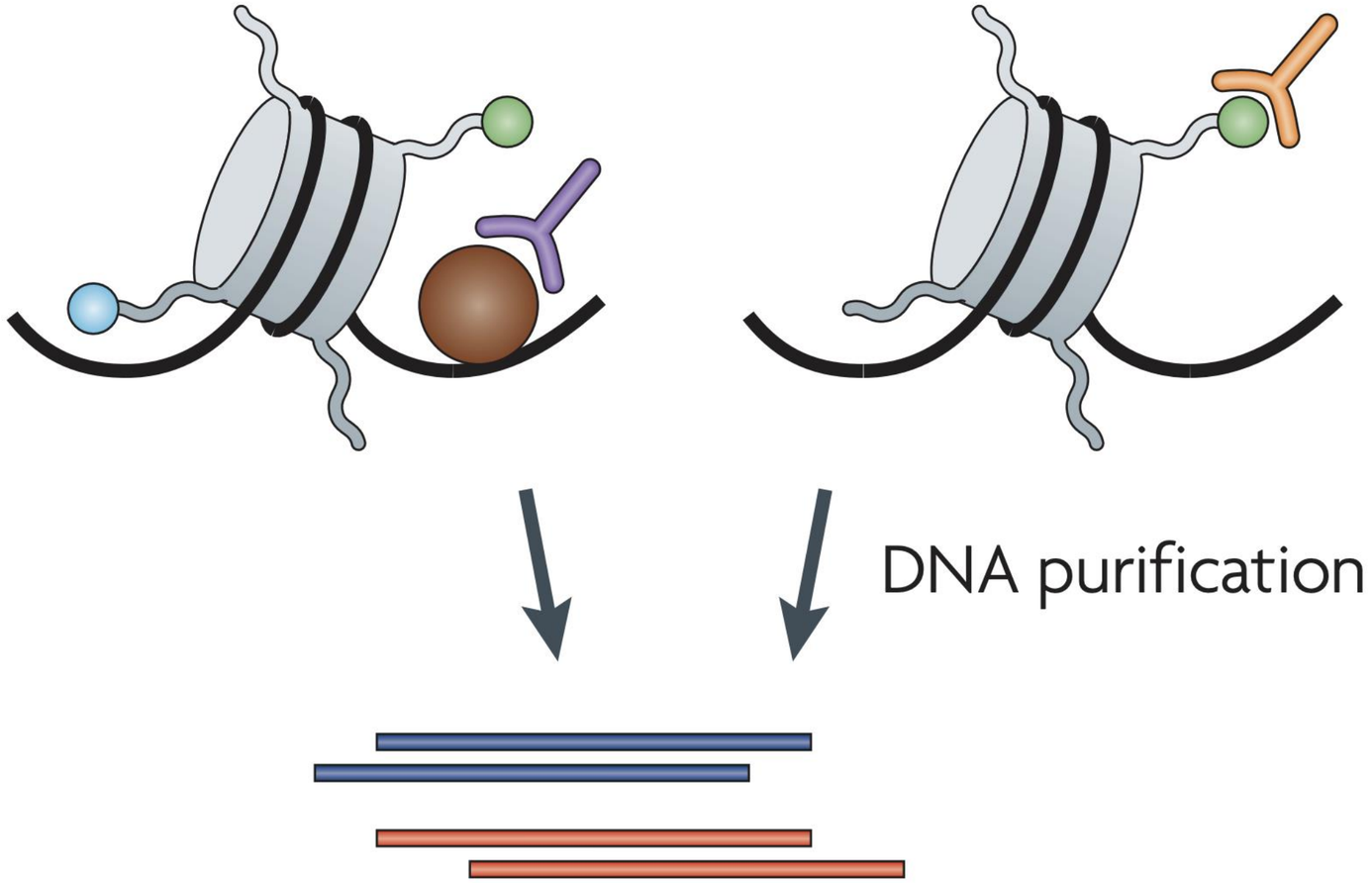
Adapted from Park (2009). Nature Reviews Genetics.

Fragmented, protein-bound DNA is immunoprecipitated using specific antibodies



Adapted from Park (2009). Nature Reviews Genetics.

Cross-linking is reversed and DNA is purified for sequencing

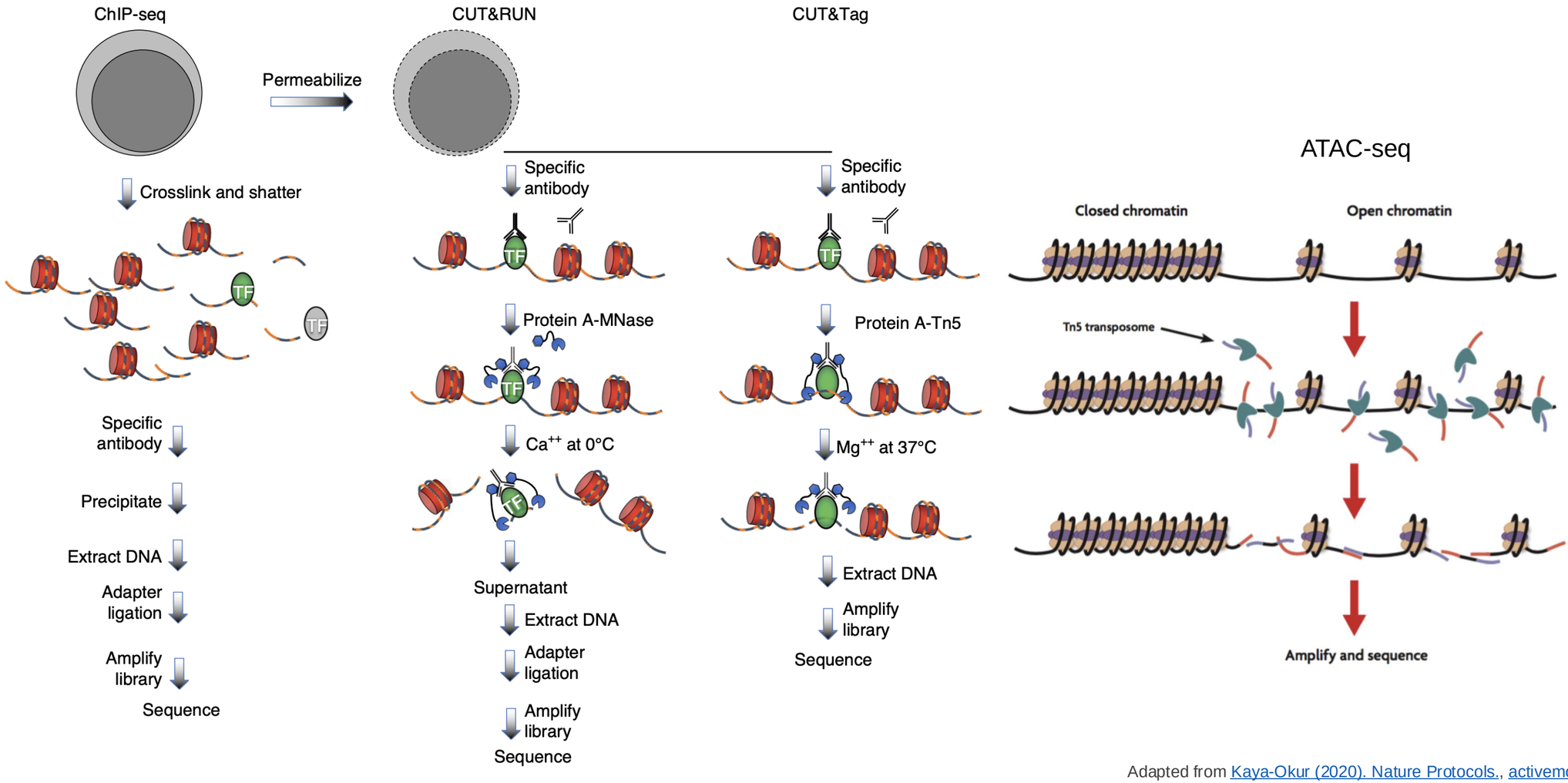


Adapted from Park (2009). Nature Reviews Genetics.

ChIP-PCR vs. ChIP-chip vs. ChIP-Seq

ChIP-PCR	ChIP-chip	ChIP-Seq
• Targeted regions of genome • Regions known beforehand • Cheaper • More time efficient • qPCR can allow quantitative comparisons • qPCR can confirm ChIP successful	• Whole genome (but can profile specific regions) • Microarray-based • 30-100 bp resolution typically • Requires ~a few micrograms DNA • Useful for broad binding	• Whole genome • Next-generation sequencing • Single nucleotide resolution • Only requires ~10 – 50 ng of DNA • Becoming more cost effective • Useful for sharp binding

Alternative methods to ChIP-Seq



Adapted from Kaya-Okur (2020). Nature Protocols., activemotif.com/blog-atac-seq

Submitting Samples for Sequencing and Analysis

Submitting ChIP-Seq samples for Hartwell sequencing

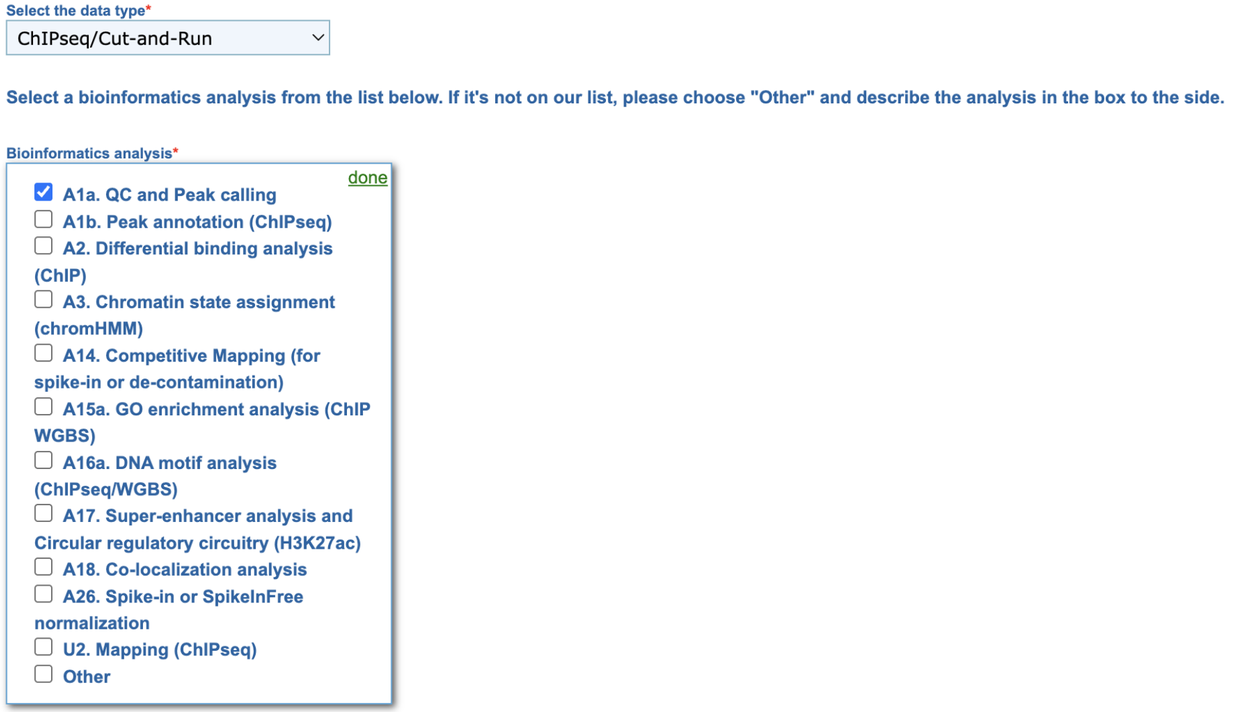
- Submit request for Hartwell sequencing via SRM
- Fill out template spreadsheet to submit (or manually file out table in SRM)
  - Sample info for each well of 96 well plate
- Hartwell pipeline is optimized for 10 ng input DNA per sample, 1 ng input accommodated, 100 pg attempted (submit in 52 uL volume)
- Receive email with SRM order confirmation with QR for sample label
- After sequencing, receive email with fastq file paths, basic sequencing QC

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	**Please use this excel to upload samples for Genome Sequencing Service in SRM2 System**															
3	**Well Location**	**Sample Name**	**Is this a Human Derived Sample?**	**SJ Tissue Bank #**	**SJUID**	**Alternative # (lab, cooperative group, etc.)**	**Submission Material**	**Xenograft?**	**Application**	**Illumina Sequencer**	**Run Type**	**Read Length**	**Molecules Sequenced**	**Reference Genome**	**Please specify Reference Genome**	**User Comments**
4	**A01**								ChIP-seq	NovaSeq	Single End	50 bps	Default for Selected Application			
5	**B01**															
6	**C01**															
7	**D01**															
8	**E01**															
9	**F01**															

Nucleic Acid for Prep and Sequencing
User Made Libraries to be Sequenced

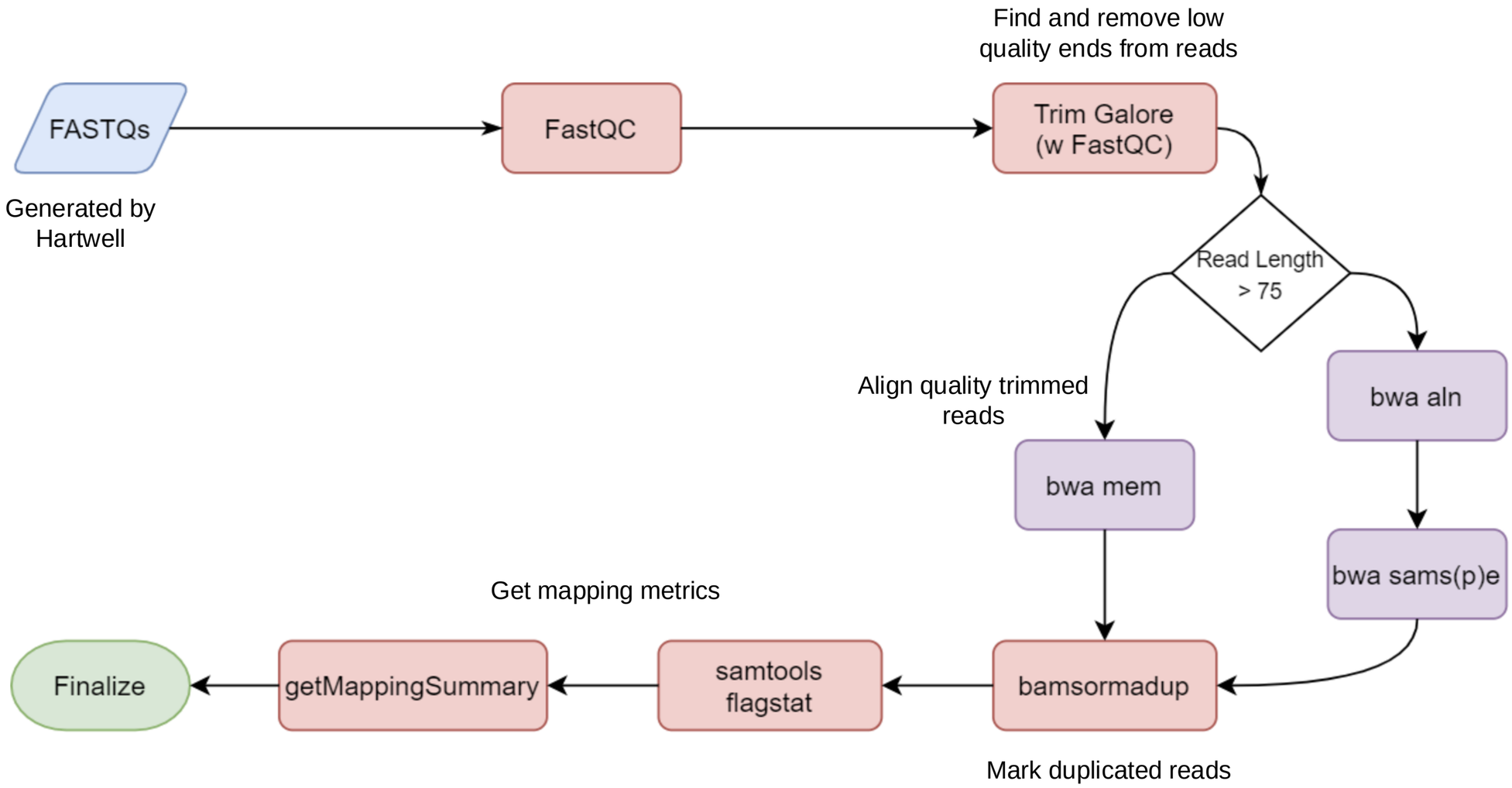
Requesting ChIP-Seq analysis from Center for Applied Bioinformatics (CAB)

- CAB AutoMapper for ChIP-Seq runs automatically after Hartwell sequencing completed
- Receive email when AutoMapper submission is started
- Receive email when AutoMapper run is completed
- AutoMapper pipeline does not include analysis, only alignment
- Must submit new SRM request for analysis
- QC and Peak Calling is standard analysis
- Additional analysis considered customized

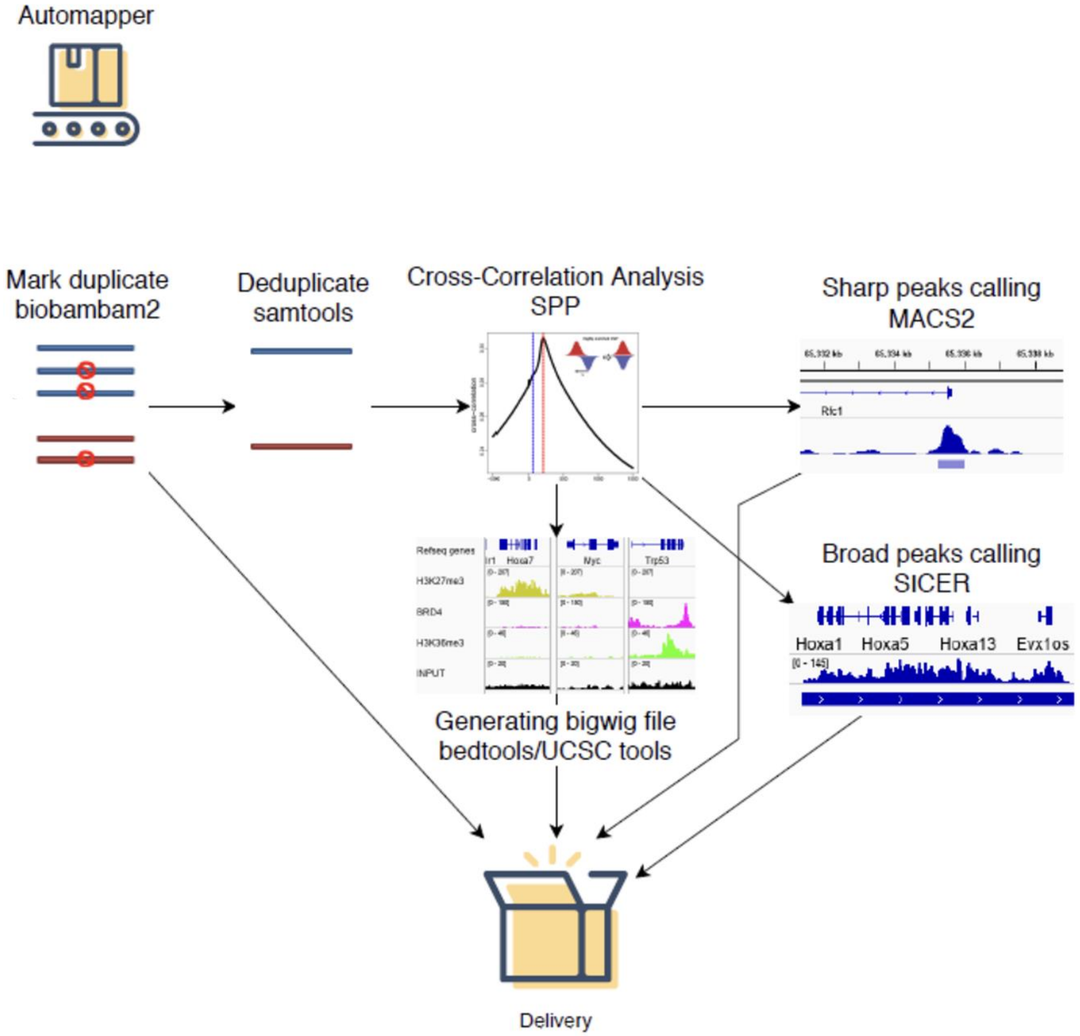


CAB ChIP-Seq AutoMapper Pipeline, QC, and Peak Calling

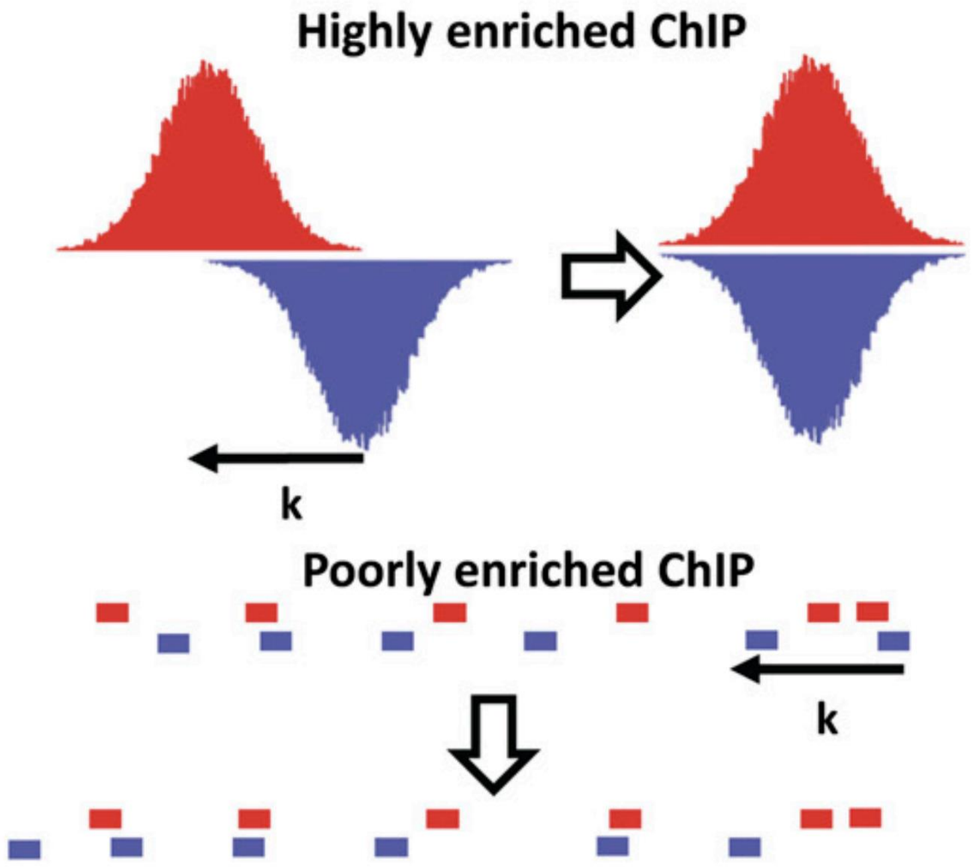
CAB AutoMapper pipeline

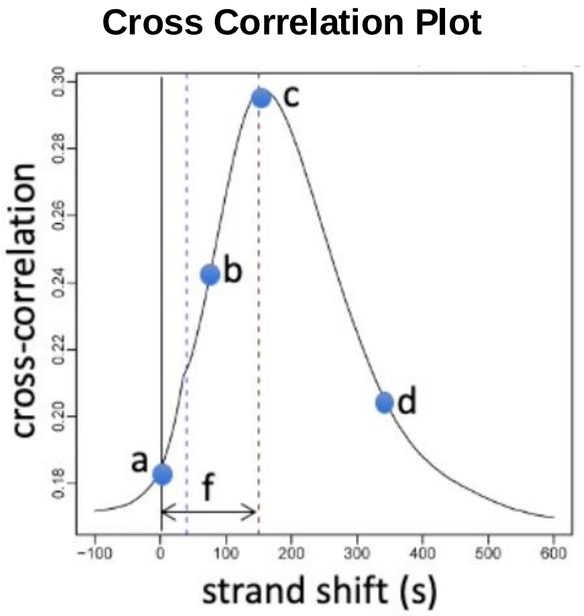


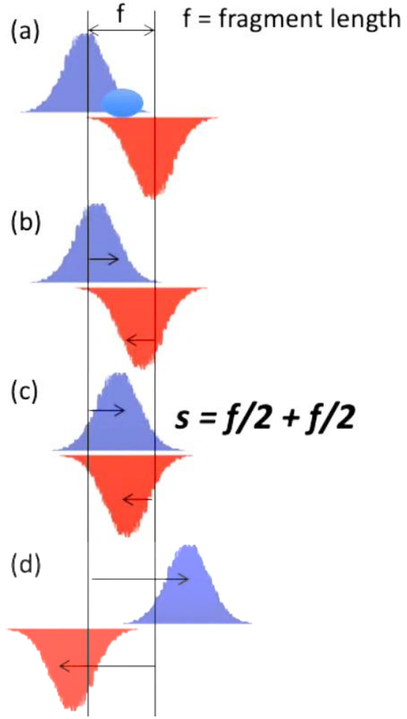
CAB QC and Peak Calling



Cross-correlation analysis indicates quality of sequencing, fragment length, binding sites

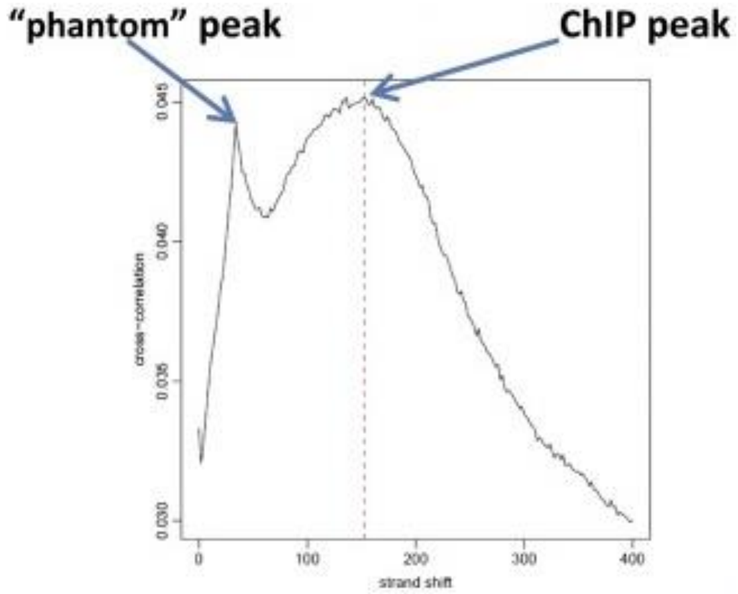


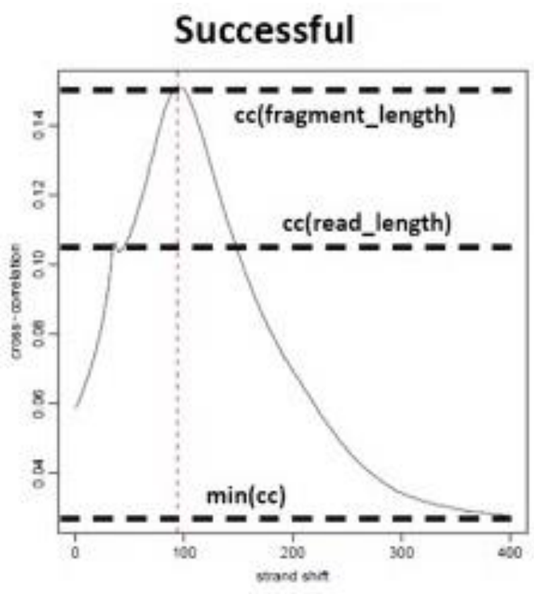


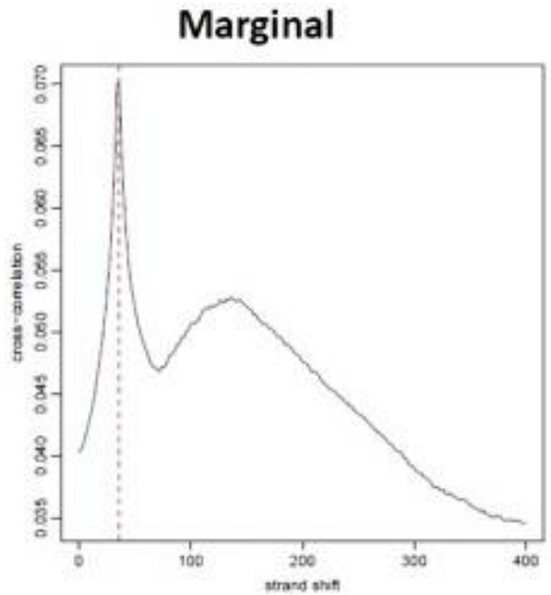


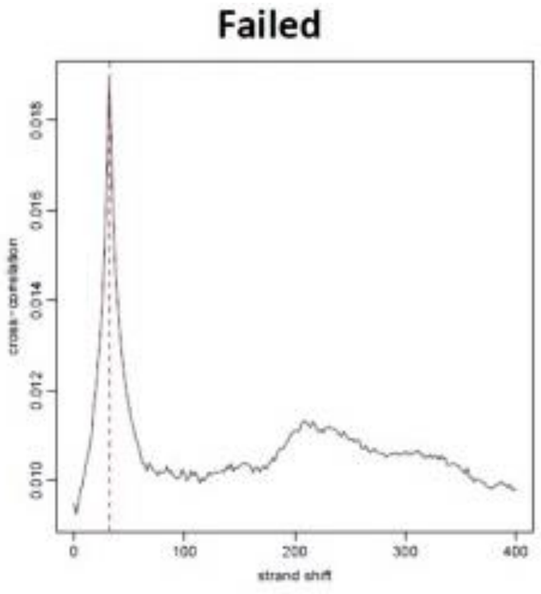
Adapted from Landt (2012). Genome Research.

Interpreting cross-correlation plots, relative stranded correlation (RSC) values









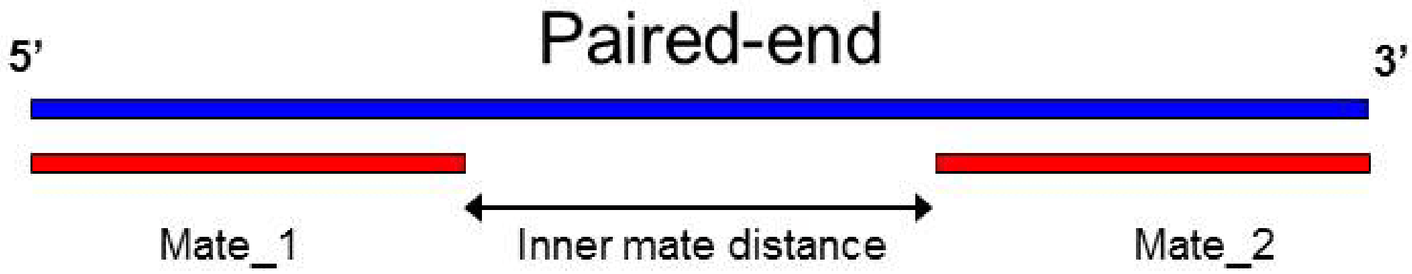
Qtag	-2	-1	0	1	2
RSC	0, 0.25	0.25, 0.5	0.5, 1	1, 1.5	≥1.5

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

Adapted from Landt (2012). Genome Research.

Cross-correlation analysis is only relevant for single-end sequencing

- If sequencing was paired end, ignore cross-correlation results
- Results are meaningless
- Paired reads will have mate read on opposite strand
- Separated by distance of ~average fragment length



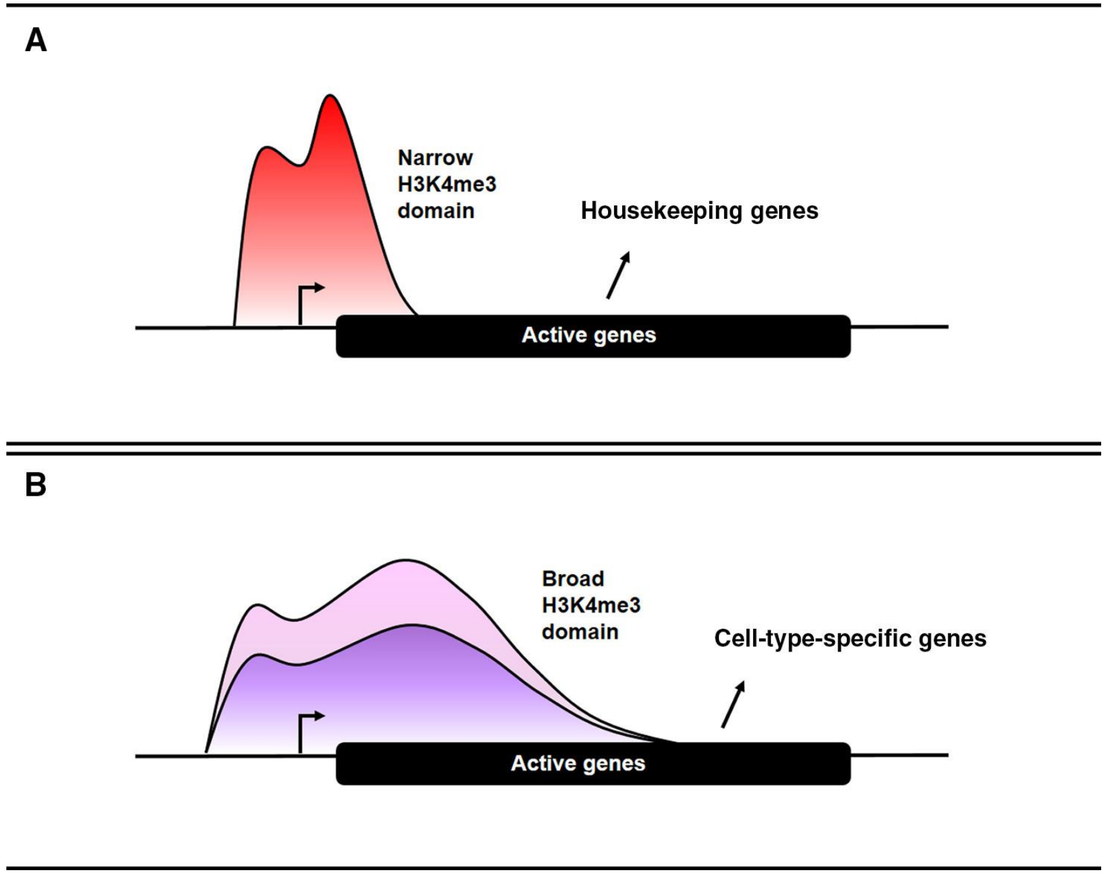
Common quality control metrics for ChIP-seq

- Mapping rate > 80%
- Duplication rate <= 30%
- Total number of unique reads (per ENCODE):
  - > 10M for narrow peaks (point-source data)
  - > 20M for broad peaks
- Fragment size > 100bp
- Qtag > 0 (single-end data only)
  - Wave pattern in cross-correlation plot, RSC invalid
- Visualize data with IGV
  - Check clear peaks
  - Known markers

Qtag	-2	-1	0	1	2
RSC	0, 0.25	0.25, 0.5	0.5, 1	1, 1.5	≥1.5

CAB pipeline can call broad or narrow peaks

- Peak calling identifies regions with enriched protein-DNA interactions
- Narrow peak calling is done with **MACS2**
- Broad peak calling is done with **SICER**
- If known, can tell CAB broad or narrow
- Or choose auto and pipeline will choose method
- Important to use correct method for peak calling

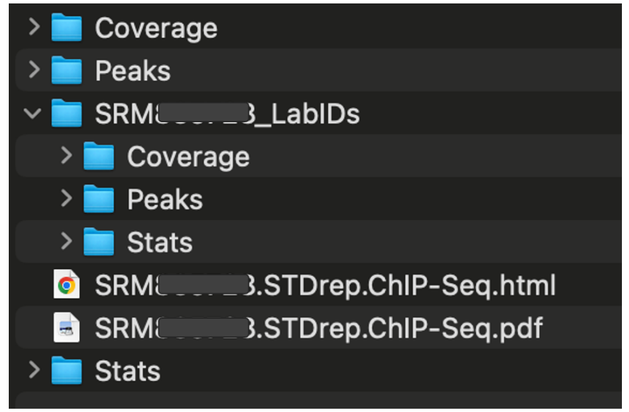


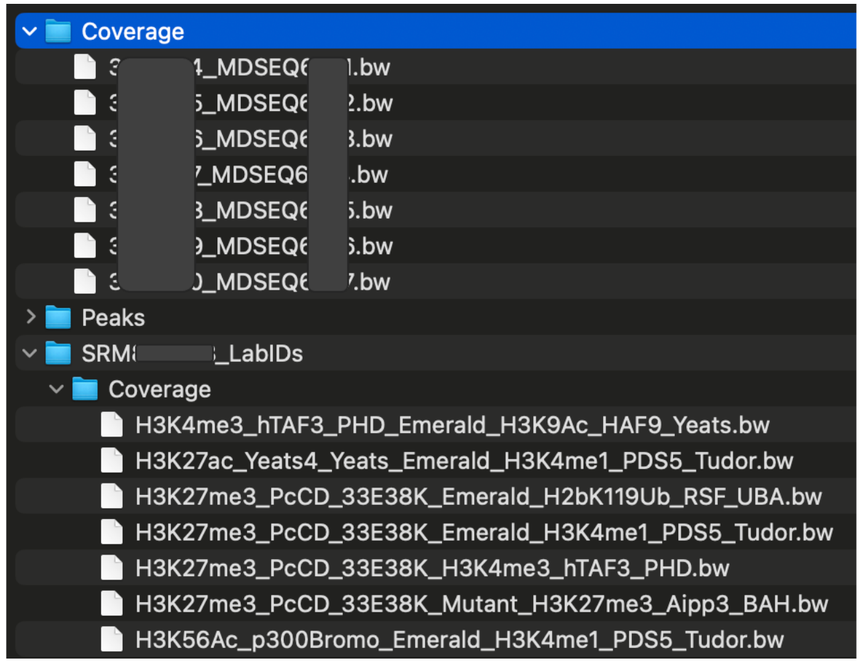
Adapted from Park (2020). The FEBS Journal.

ChIP-Seq Results Folders/Files and Report

Folder structure and file types for QC and peak calling results returned by CAB

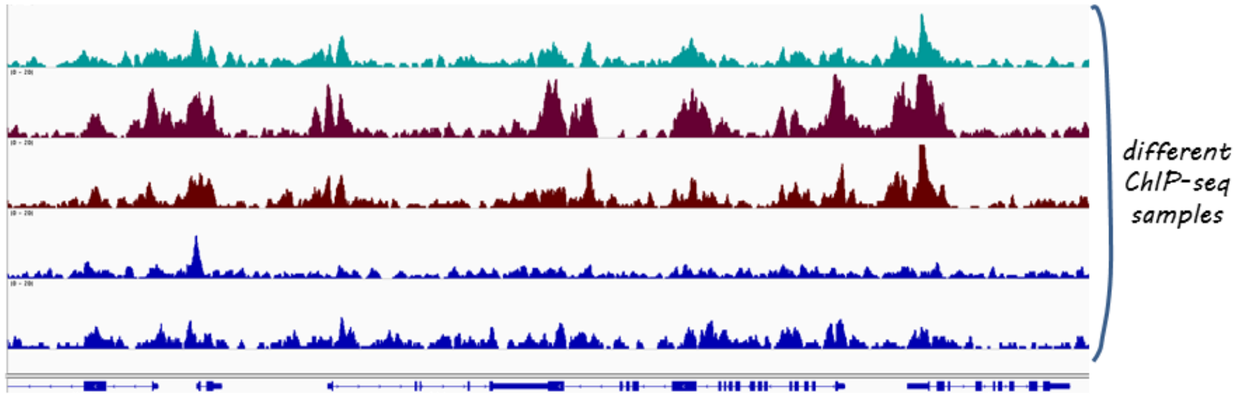
- Four folders: Coverage, Peaks, Stats, SRM#_LabIDs
- Two files: HTML report, PDF report
- Within SRM#_LabIDs: Coverage, Peaks, Stats
- SRM#_LabIDs contains files labeled by ChIP targets
- Coverage folders will contain BigWig files
  - Use these to visualize data in IGV, etc.
- Peaks folders will contain peak calling results
  - Use these to visualize peaks in IGV, etc.
  - Contain “filter” version of each peak files
  - Filter files remove ENCODE “blacklist” regions
- Stats folders will contain four QC-related files
  - Mapping metrics, QC metrics
  - Cross-correlation analysis table
  - Cross-correlation plot





Common options for visualizing data

- Integrative Genomics Viewer (IGV) - download desktop app or use web browser
- UCSC Genome Browser – select reference genome, upload your files (custom tracks)
- St. Jude ProteinPaint – select reference genome, upload your files
- Files to view often includes BigWig, broadPeak, narrowPeak, bam files with expression data (e.g. RNA-Seq)



Adapted from hbctraining.github.io/Intro-to-ChIPseq

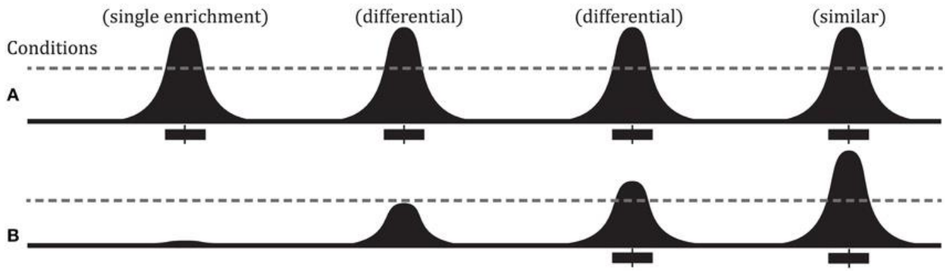
Example ChIP-Seq report from CAB

- Interactive HTML report, static PDF report
- HTML, PDF examples of reports available on CAB’s wiki page
- Walk through HTML example
- Download HTML to follow along here

Additional ChIP-Seq Analyses from CAB

Examples of other analyses that can be requested from CAB: Differential binding

- Additional analyses are considered customized or collaborative
- Require additional SRM requests
- For example:
  - Differential binding site analysis
  - Chromatin state assignment
- Differential binding site analysis can identify differences in enrichment peaks across conditions



Select the data type*

ChIPseq/Cut-and-Run

Select a bioinformatics analysis from the list below. If it's not on our list, please choose "Other" and describe the analysis in the box to the side.

Bioinformatics analysis*

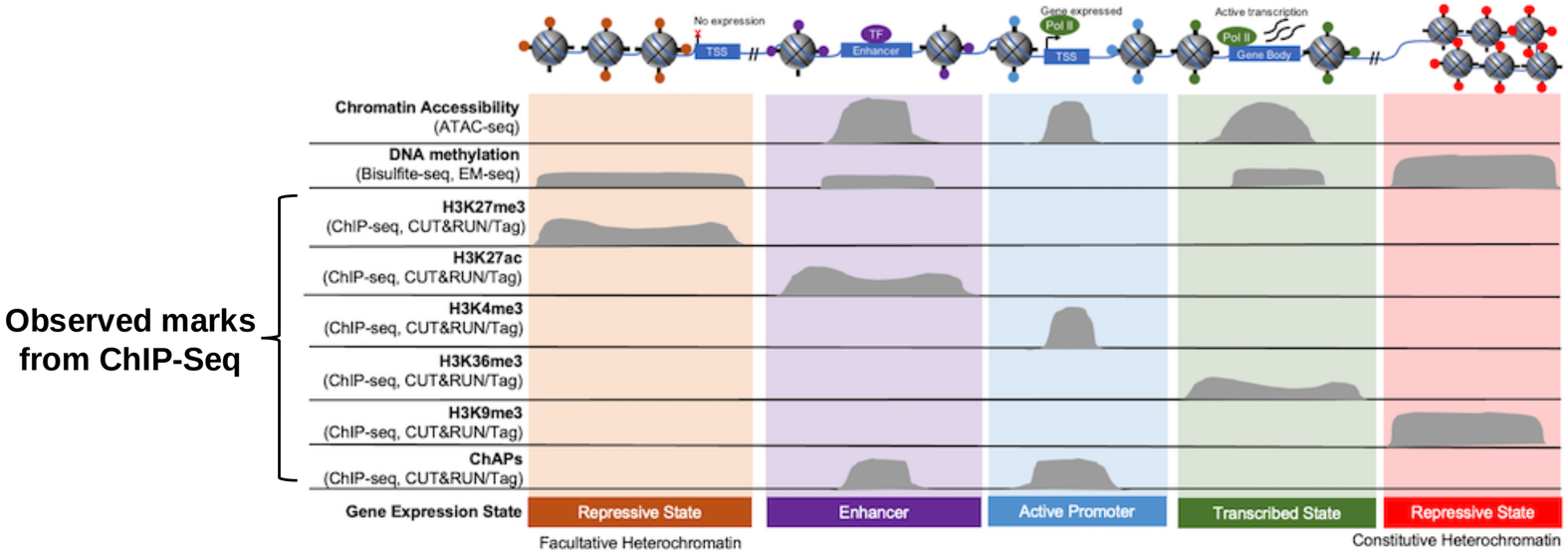
done

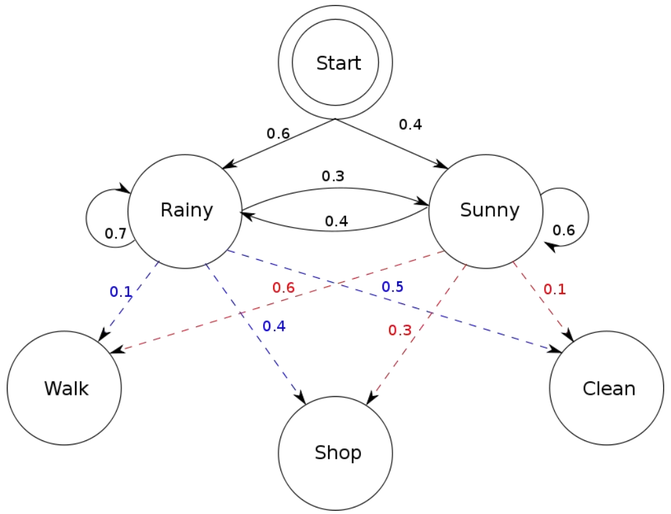
- A1a. QC and Peak calling
- A1b. Peak annotation (ChIPseq)
- A2. Differential binding analysis (ChIP)
- A3. Chromatin state assignment (chromHMM)
- A14. Competitive Mapping (for spike-in or de-contamination)
- A15a. GO enrichment analysis (ChIP WGBS)
- A16a. DNA motif analysis (ChIPseq/WGBS)
- A17. Super-enhancer analysis and Circular regulatory circuitry (H3K27ac)
- A18. Co-localization analysis
- A26. Spike-in or SpikeInFree normalization
- U2. Mapping (ChIPseq)
- Other

Adapted from Wu (2015). Frontiers in Genetics.

Examples of other analyses that can be requested from CAB: Chromatin state assignment

- Chromatin state assignment uses ChromHMM to predict state
  - Based on Hidden Markov Model (HMM), models presence or absence of chromatin marks, annotates genome
- Hidden Markov Models are probabilistic, predict “outcomes” based on observable parameters
- Includes “hidden” states that influence outcome but aren’t “observable”
- In this case, “outcome” is chromatin state, predicted based on observed chromatin mark patterns





Credit: epicypher.com

Questions?