# ChIP-Seq Data Analysis: Probing DNA-Protein Interactions

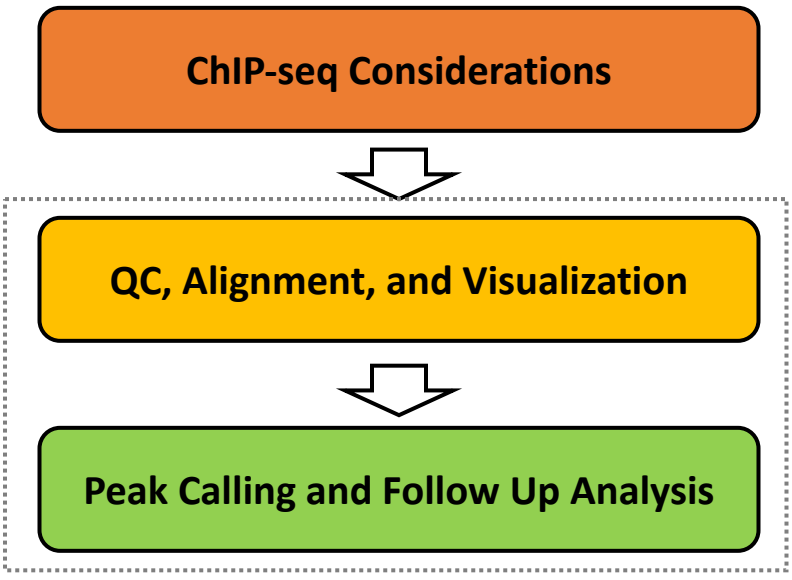
Paul Schaughency[1,2], Tovah Markowitz[1], Vishal Koparde[3]

**Schedule**

| | |
|---|---|
| 9:30 - 10:15 | Introduction to ChIP-Seq |
| 10:15 - 10:30 | Q&A |
| 10:30 - 11:20 | QC, Alignment, and Visualization |
| 11:20 - 12:00 | Peak Calling and Follow Up Analysis |
| 12:00 - 12:30 | Q&A |

[1]NIAID Collaborative Bioinformatics Resource (NCBR), [2]Center for Cancer Research Sequencing Facility (CCR-SF) Bioinformatics, [3]Center for Cancer Research Collaborative Bioinformatics Resource (CCBR)

1

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**ChIP-seq Considerations**

**Introduction**

**Best Practices**

**Frustrations of ChIP-seq**

**Orthogonal Methods**

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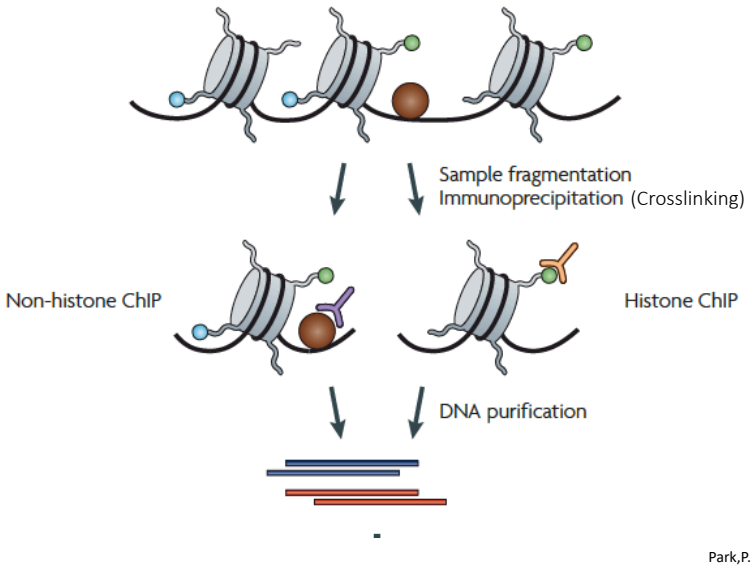
## What is Chromatin ImmunoPrecipitation (ChIP)?




Park,P. Nature Reviews. 2009.

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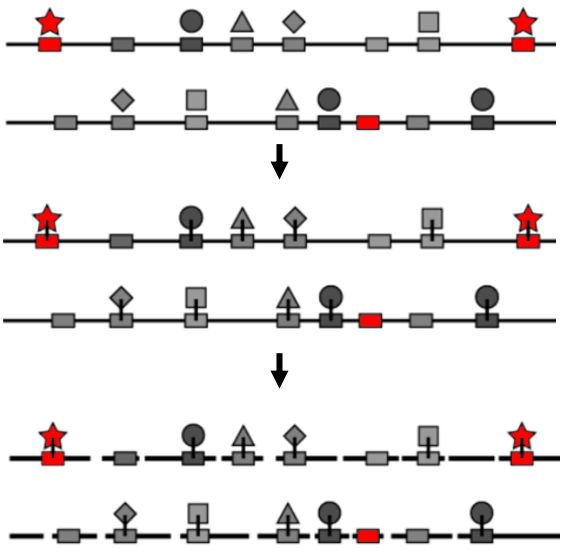
# What is ChIP?



DNA

Crosslinking

Fragmentation

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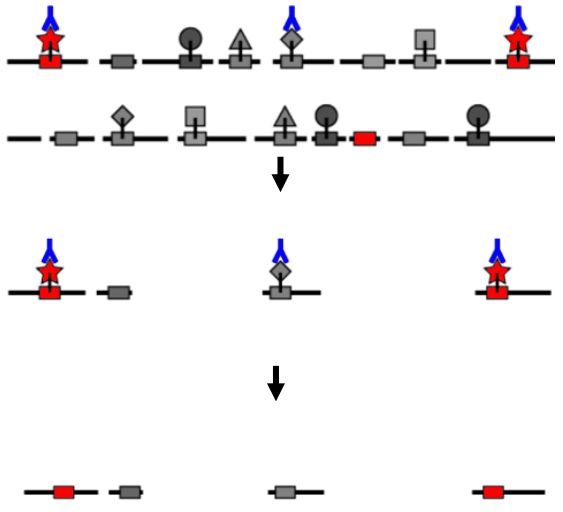
# What is ChIP?



Antibody Binding

Enrichment

Reverse Crosslinking

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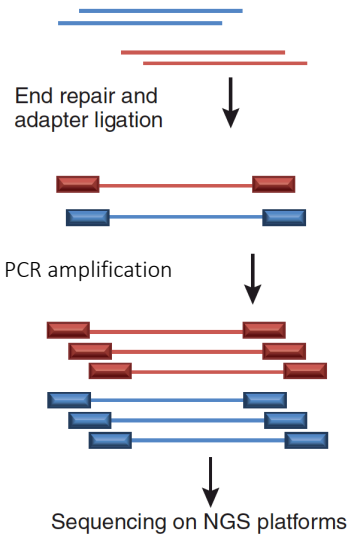
# What is ChIP-seq?




Kidder et. al. Nature Immunology. 2011

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# Why do ChIP-seq?

- Define Protein-DNA interactions/Histone modifications across the entire genome and different conditions.
- Define DNA binding sites for DNA-binding proteins.
- Reveal gene regulatory networks when combined with RNA-seq and/or Methylation data.

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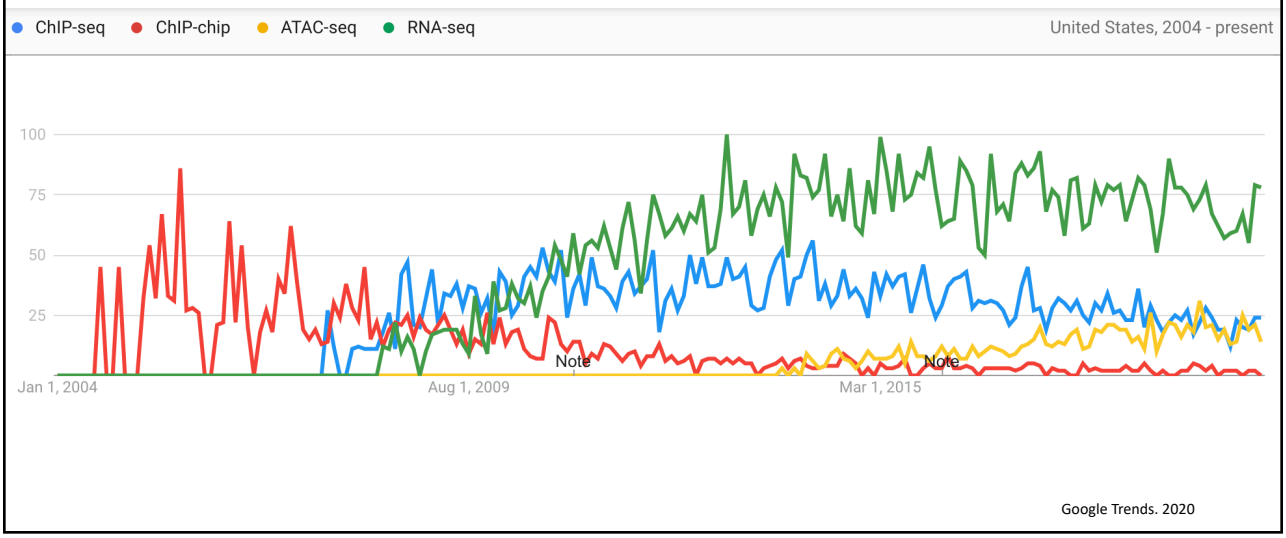
# Utilization of ChIP-seq




Google Trends. 2020

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# Adaptations to ChIP-seq

- ChIP-Exo
  - Adds two exonuclease steps to increase the resolution of ChIP
- X-ChIP-seq
  - ChIP-seq with MNase not sonication.
- Highthroughput ChIP (HT-ChIP)
  - ChIP for up to 96 antibodies at once.
- Single cell ChIP (scChIP)
  - ChIP on each individual cell.

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# And others…

- AHT-ChIP-Seq
- BisChIP-Seq
- CAST-ChIP
- ChIP-BMS
- ChIP-BS-seq
- ChIPmentation
- Drop-ChIP
- Mint-ChIP
- PAT–ChIP
- reChIP-seq

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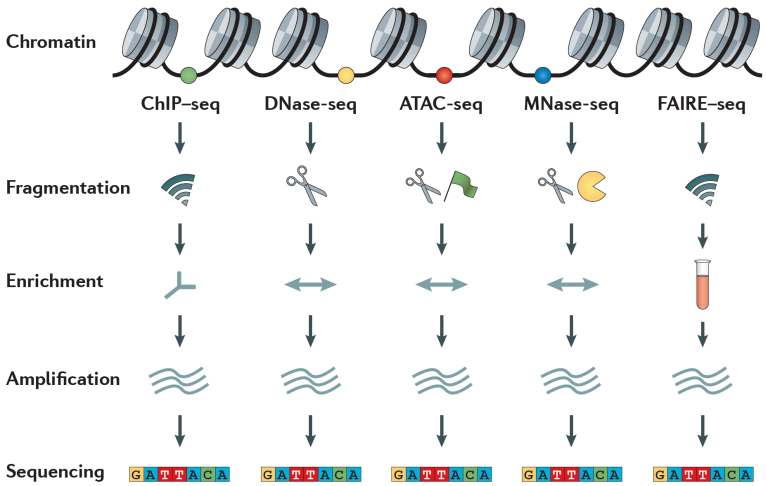
# Alternative methods to find open chromatin




Meyer and Liu. Nature Reviews. 2014.

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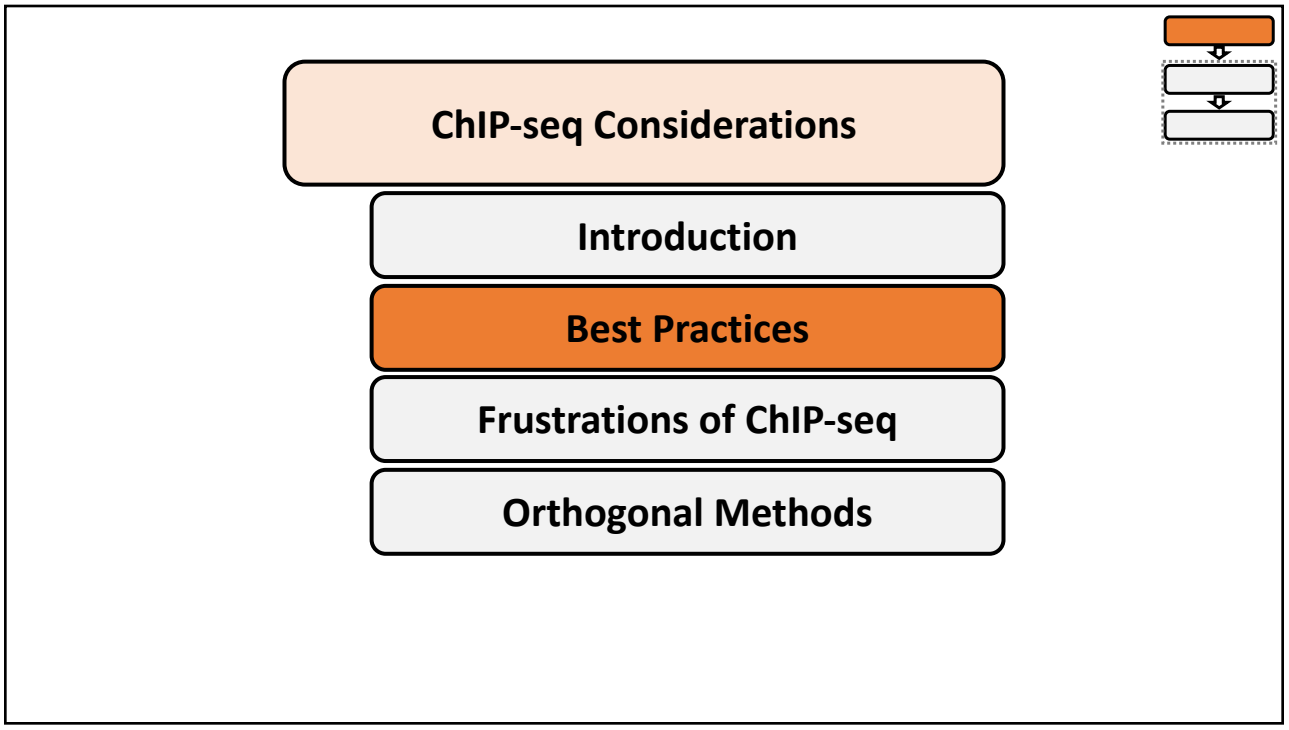


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## ENCODE best practices

- "ChIP-seq grade" antibodies
- Control samples created in parallel with matching ChIP samples
- At least 2 biological replicates
- Minimum useable reads/fragments
  - Useable reads: uniquely mapped after removal of PCR duplicates
  - For Transcription Factor or other narrow features: 10-15M
  - For Histone or other broad features: > 30M
- No preference between single-end or paired-end sequencing

https://www.encodeproject.org/about/experiment-guidelines/

Landt et al 2012. Genome Res

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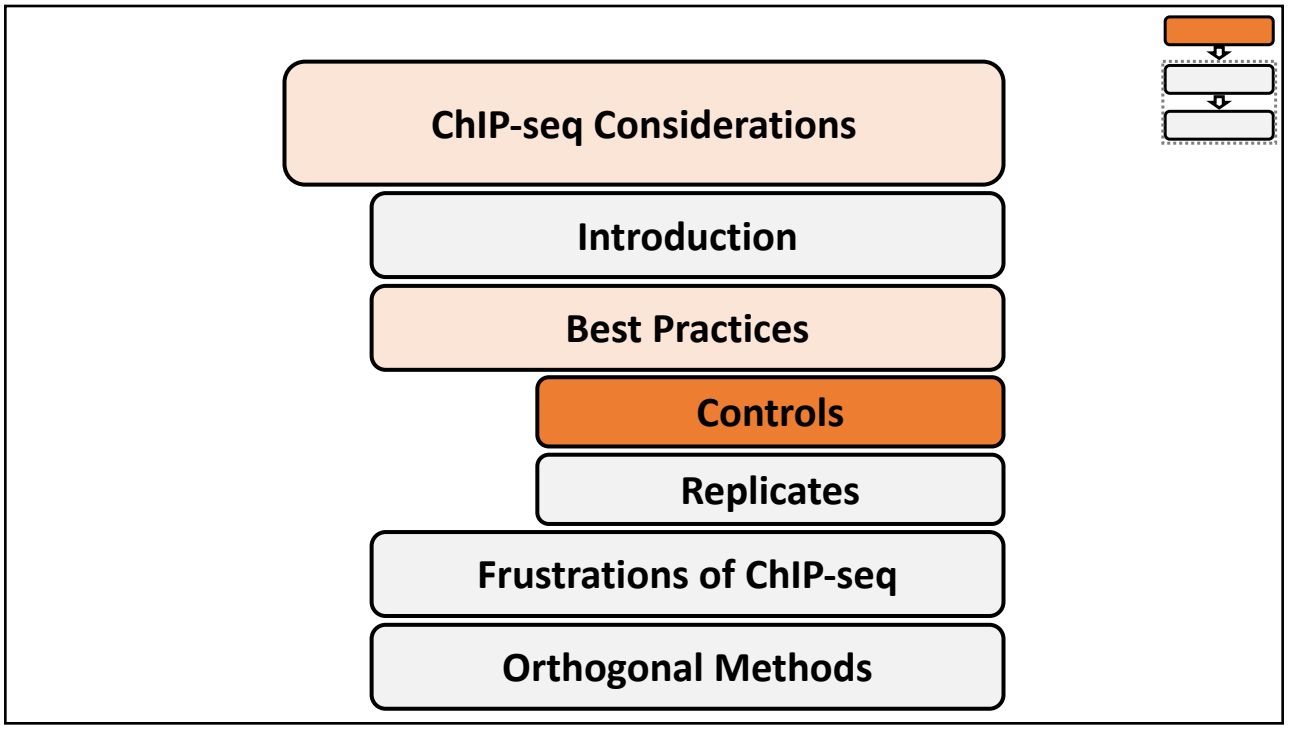


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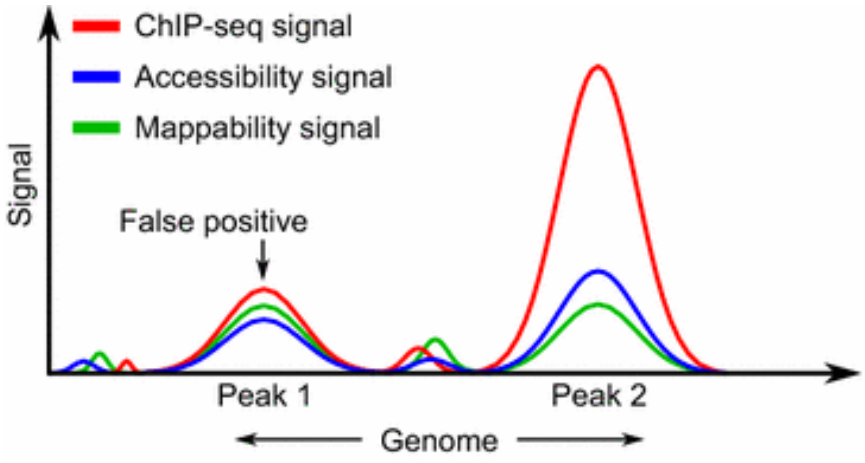
## Why you need controls




Ramachandran et al 2015. BMC Epigenetics & Chromatin

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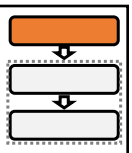
## Biases controls can correct for:



- Copy number variation
- Incorrect mapping of repetitive regions
- GC bias
- Non-uniform fragmentation
- Non-specific pull-down

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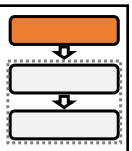
## Types of controls



- Input:
  - Crosslink, lyse, and fragment like ChIP but no IP step
- Mock (sometimes also referred to as IgG):
  - Processed like a ChIP sample, but IP without an antibody (just the beads)
- IgG:
  - ChIP with an antibody that has no target within the nucleus of the cells of interest

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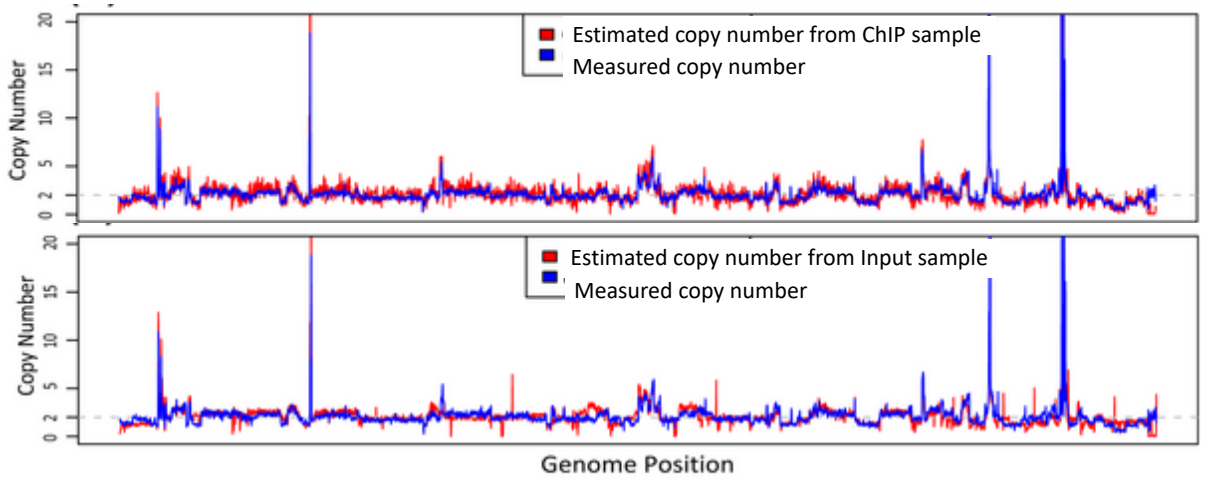
# Copy number biases




Vega et al 2009. PLOS One

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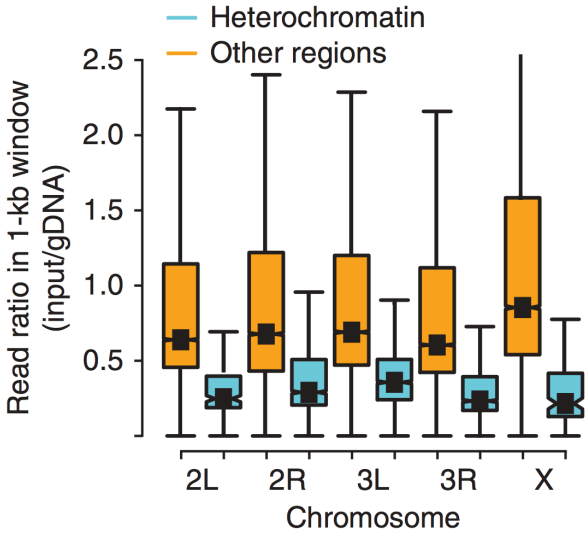
# Heterochromatin biases




Chen et al 2012. Nature Methods

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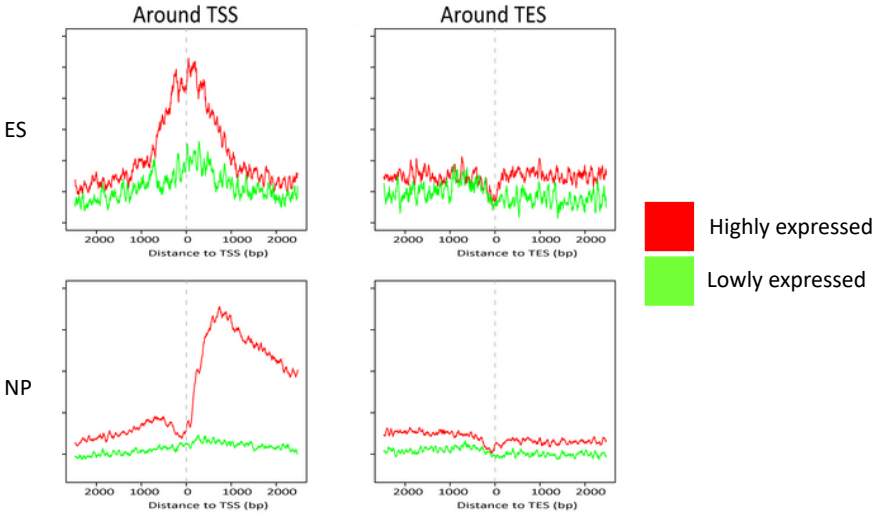
# Biases vary by cell type and are affected by gene expression




Vega et al 2009. PLOS One

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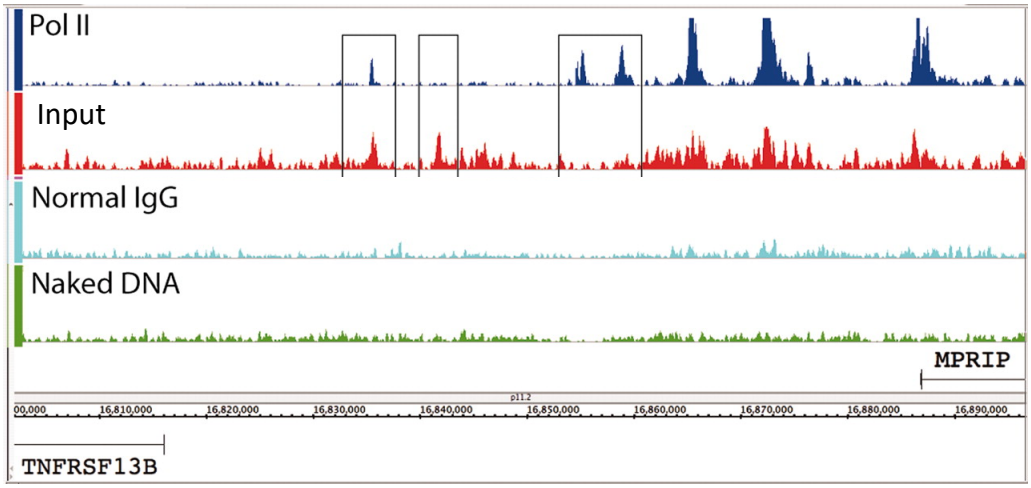
# Different controls behave differently




Auerbach et al 2009. PNAS

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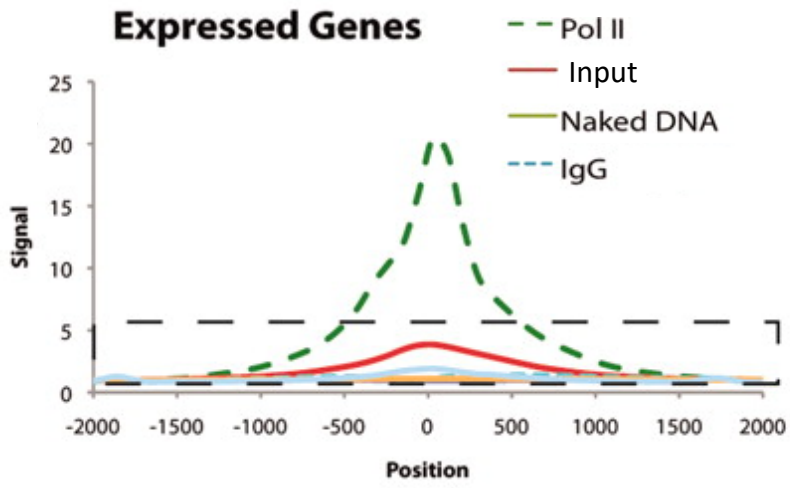
# Input is enriched at open chromatin while IgG is not




Auerbach et al 2009. PNAS

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## ChIP-seq Considerations

- Introduction
- Best Practices
- Controls
- **Replicates**
- Frustrations of ChIP-seq
- Orthogonal Methods

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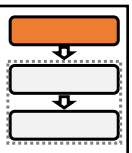
# Types of replicates



- Biological/Experimental
  - To capture variability between different runs
  - eg, repeating ChIP multiple times with the same antibody with cells from the same line grown separately, starting with a different passage of cells, or related samples with the same mutation of interest
- Technical
  - Often refers to resequencing the same libraries to deal with sequencing biases
  - Can also include replication of any/all steps following fixation

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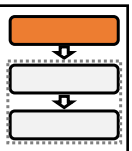
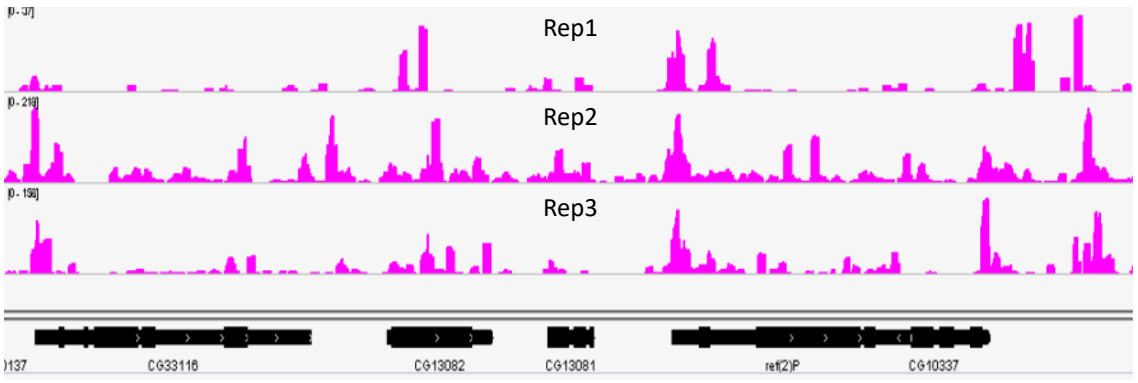
# Biological/Experimental replicates are needed to differentiate between real peaks and background noise






Yang et al 2014. Comput Struct Biotechnol J

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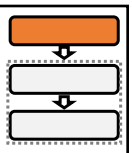
# Dealing with replicates



| | Number of Samples | Information from individual replicate |
|---|---|---|
| Pooling all replicates | No limitation | Lost |
| Merge after peak calling | Pairwise combinations | Kept |
| Select one best replicate | No limitation | Lost |
| Majority rule | No limitation | Kept |

Yang et al 2014. Comput Struct Biotechnol J

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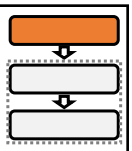
# ENCODE best practices



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- At least 2 biological replicates
- Minimum useable reads/fragments
  - Useable reads: uniquely mapped after removal of PCR duplicates
  - For Transcription Factor or other narrow features: 10-15M
  - For Histone or other broad features: > 30M
  - For input controls: > 60M is optimal (not an ENCODE best practice)
- No preference between single-end or paired-end sequencing

Landt et al 2012. Genome Res

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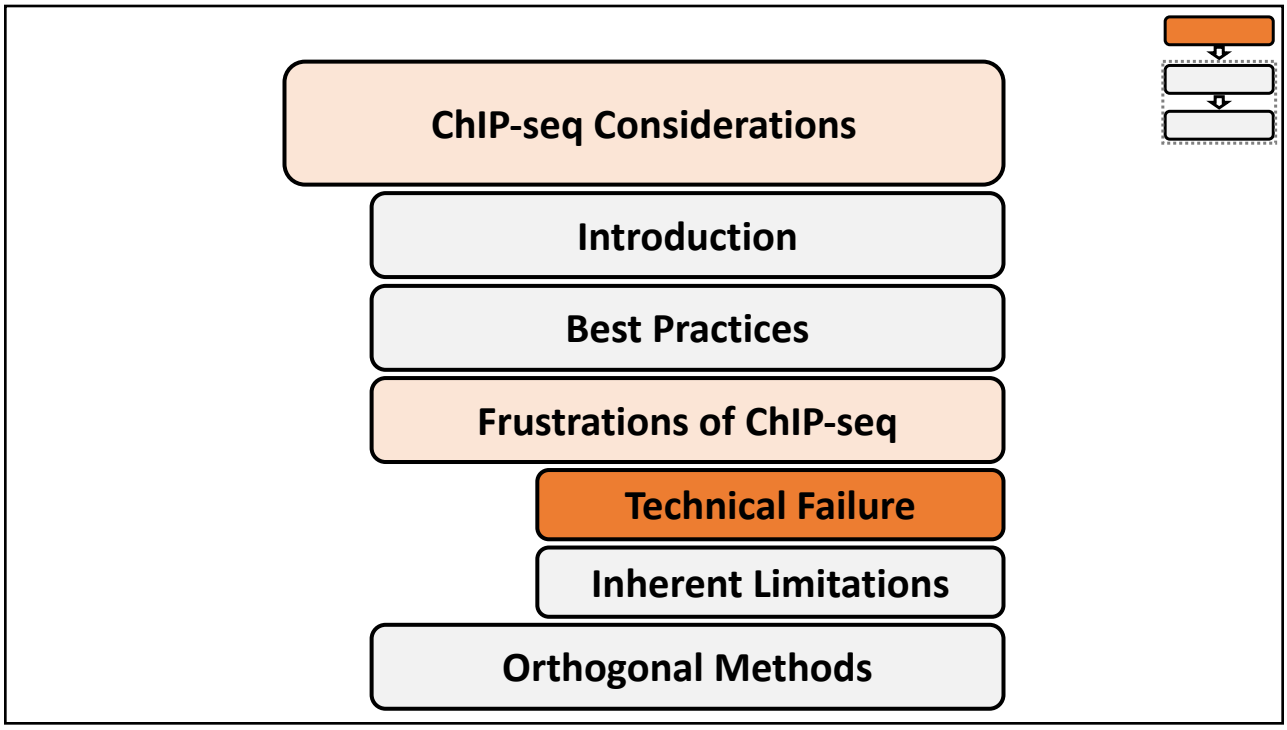
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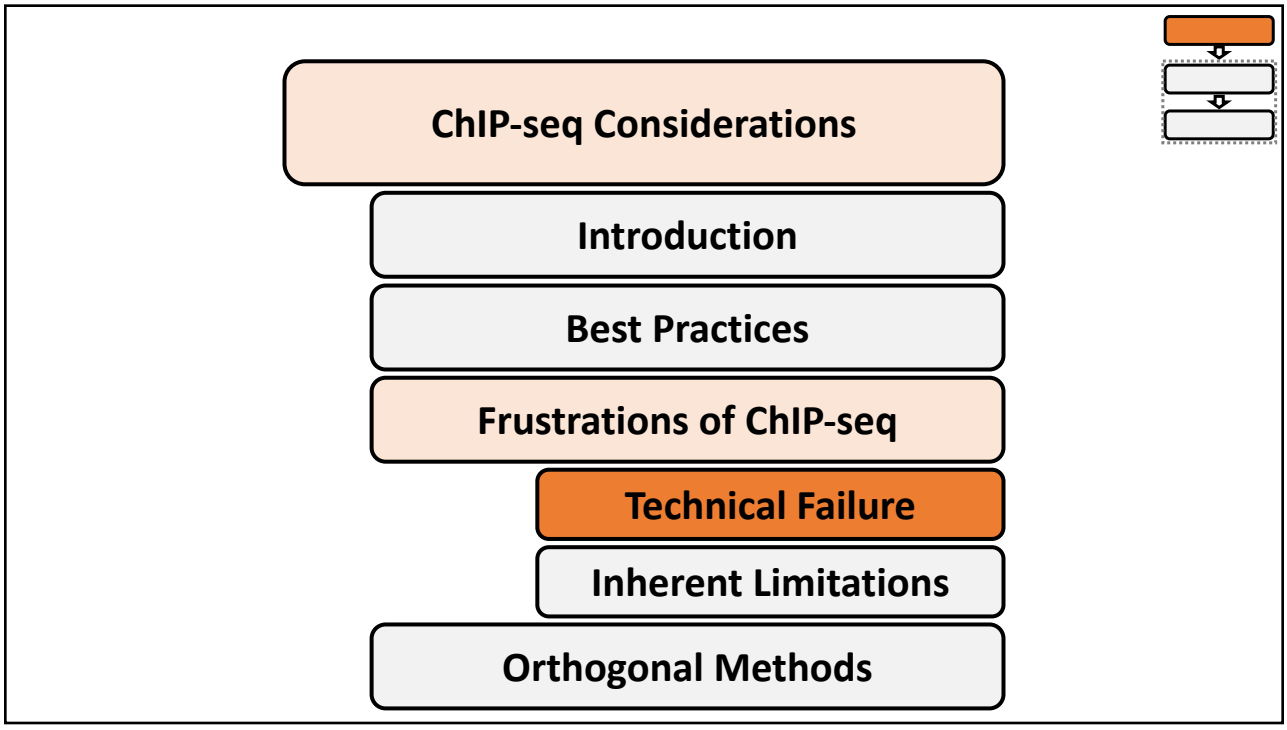
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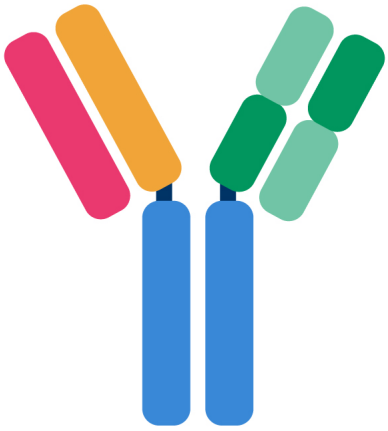
# Technical Failure: Antibodies

- Low binding affinity
- Insufficient antibody
- Non-specific binding
  - Monoclonal vs Polyclonal
- Protein is not a good ChIP candidate.



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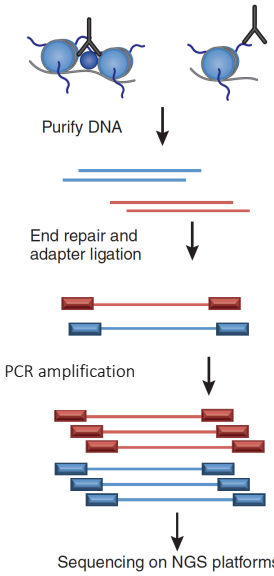
# Technical Failure: Methodological

- Not enough starting DNA
- Improper shearing/digestion
- Improper size selection
  - Illumina has a limit on insert length size.
- Too high adaptor/ChIP fragment ratio
- Too many PCR cycles




Kidder et. al. Nature Immunology. 2011

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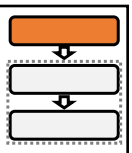
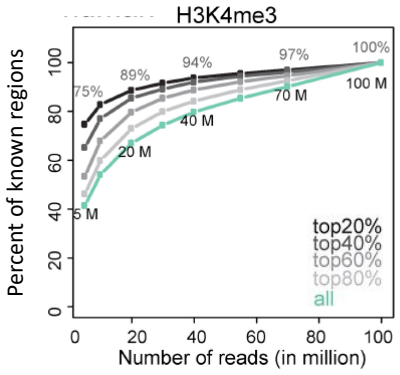
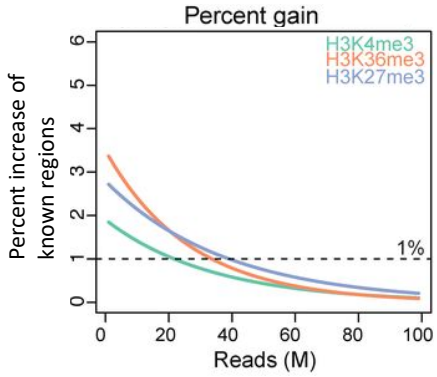
# Technical Failure: Low Sequencing Depth



- Did you saturate the amount information coming from your library?




Jung et. al. Nucleic Acids Research. 2014

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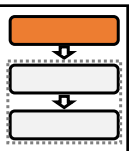
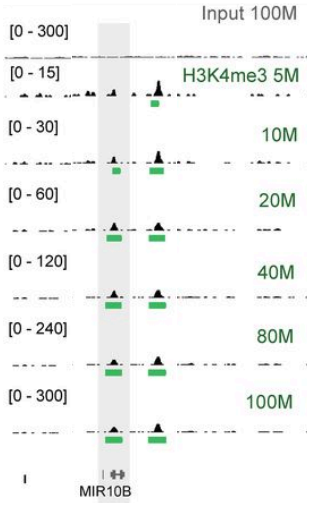
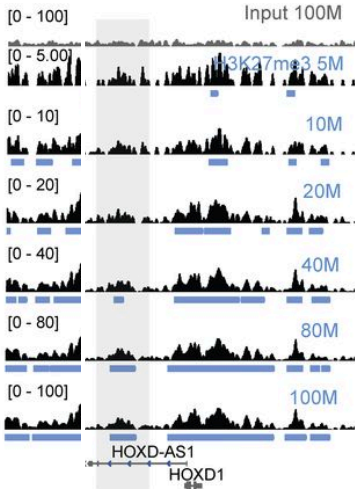
# Technical Failure: Saturation






Jung et. al. Nucleic Acids Research. 2014

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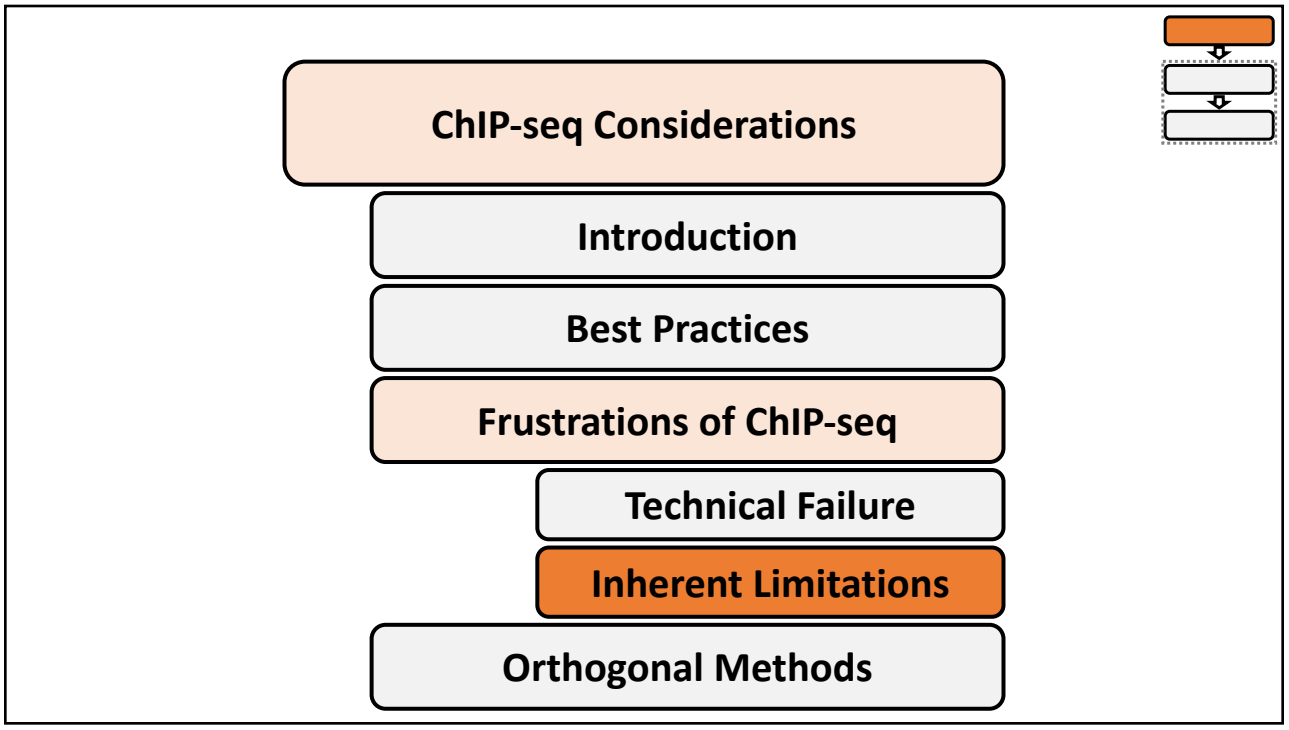


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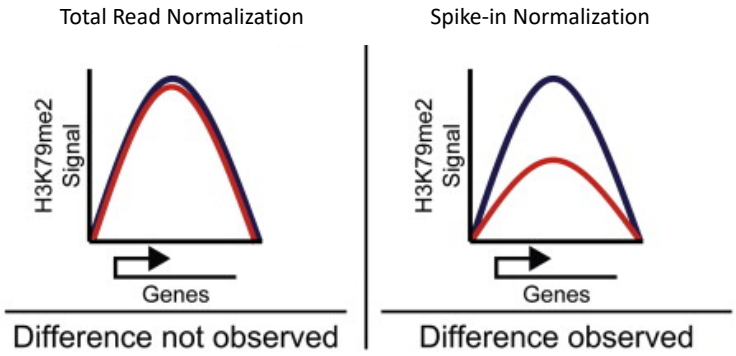
## Inherent Limitations: Relative method without spike-ins




Orlando et. al. Cell Reports. 2014.

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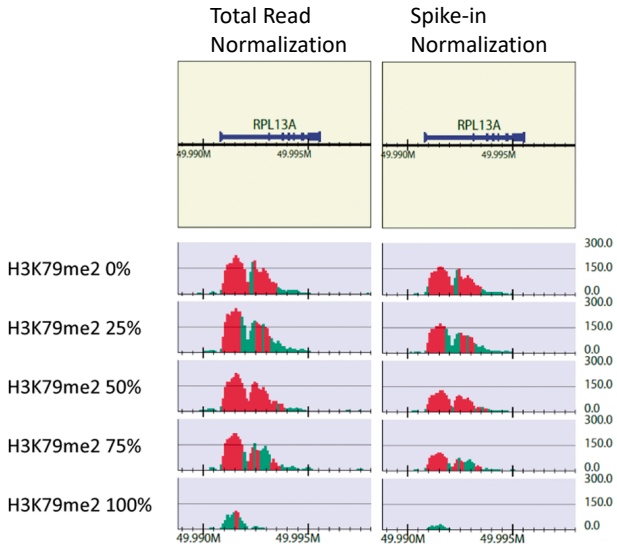


Nakato and Shirahige. Briefings in Bioinformatics. 2017.

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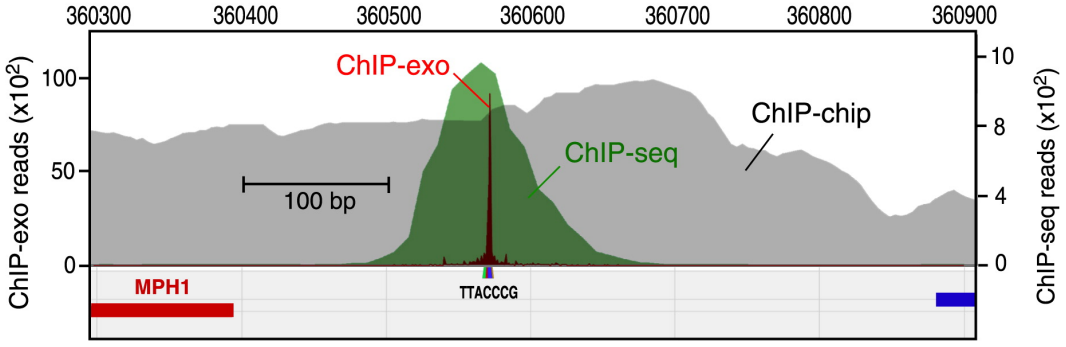
# Inherent Limitations: Resolution




Rhee and Pugh. Cell. 2011.

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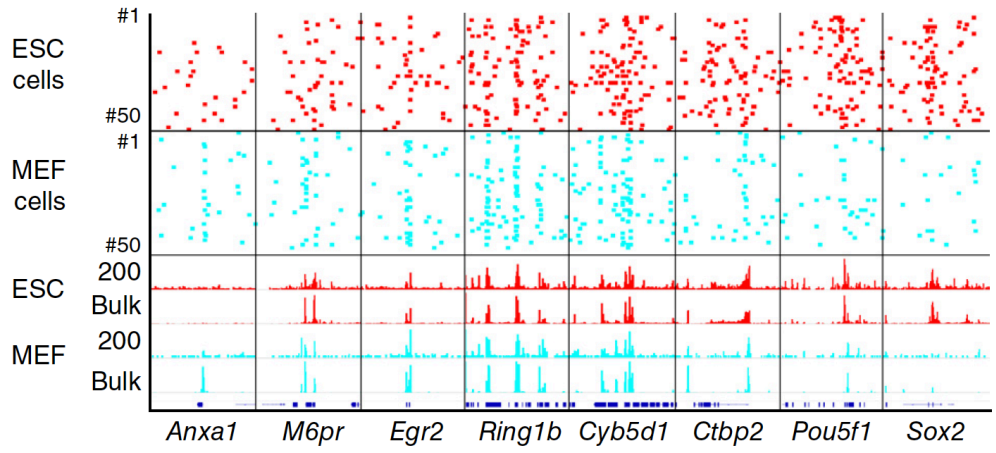
# Inherent Limitations: Bulk method not single cell




Rotem et. al. Nature Biotechnology. 2015.

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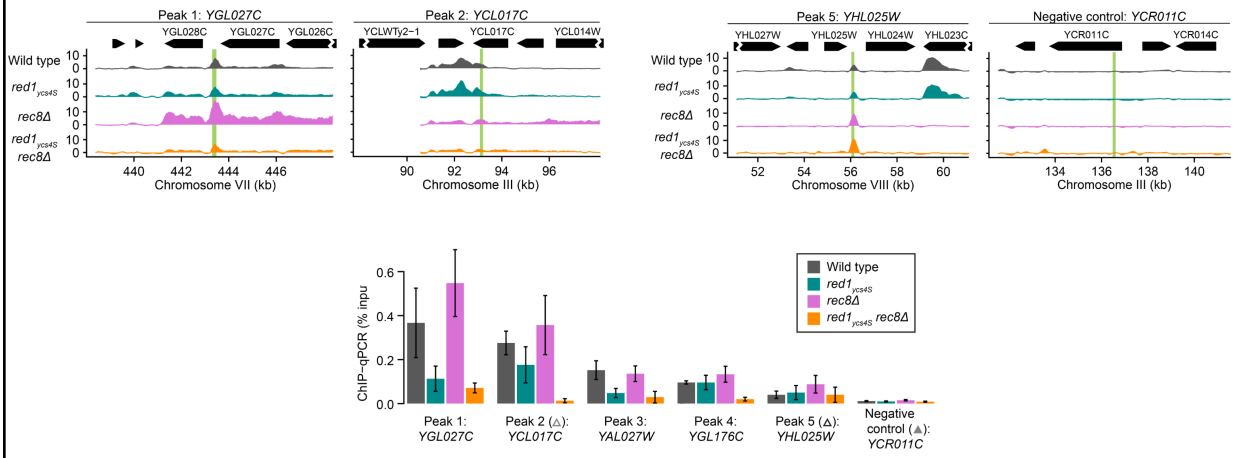
# Orthogonal Methods: ChIP-PCR/ChIP-QPCR




Markowitz et. al. PLoS Genetics. 2017.

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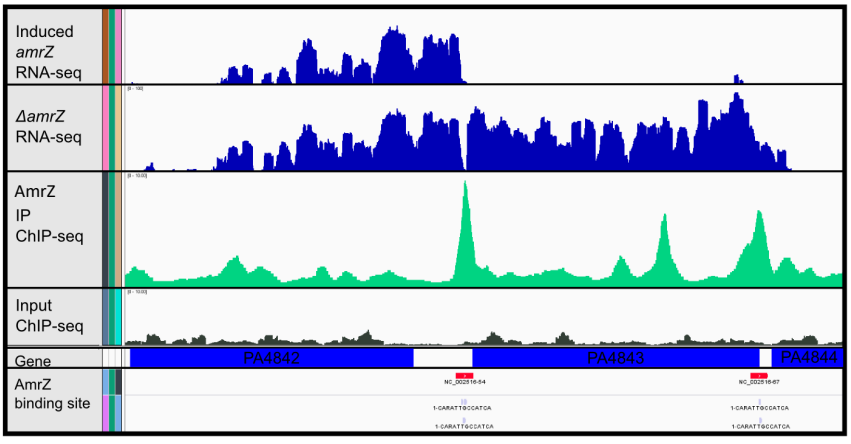
# Orthogonal Methods: Integration with other sequencing methods




ChIP-seq identifies a binding site that changes transcription when deleted

Jones et. al. PLOS Pathogens. 2014

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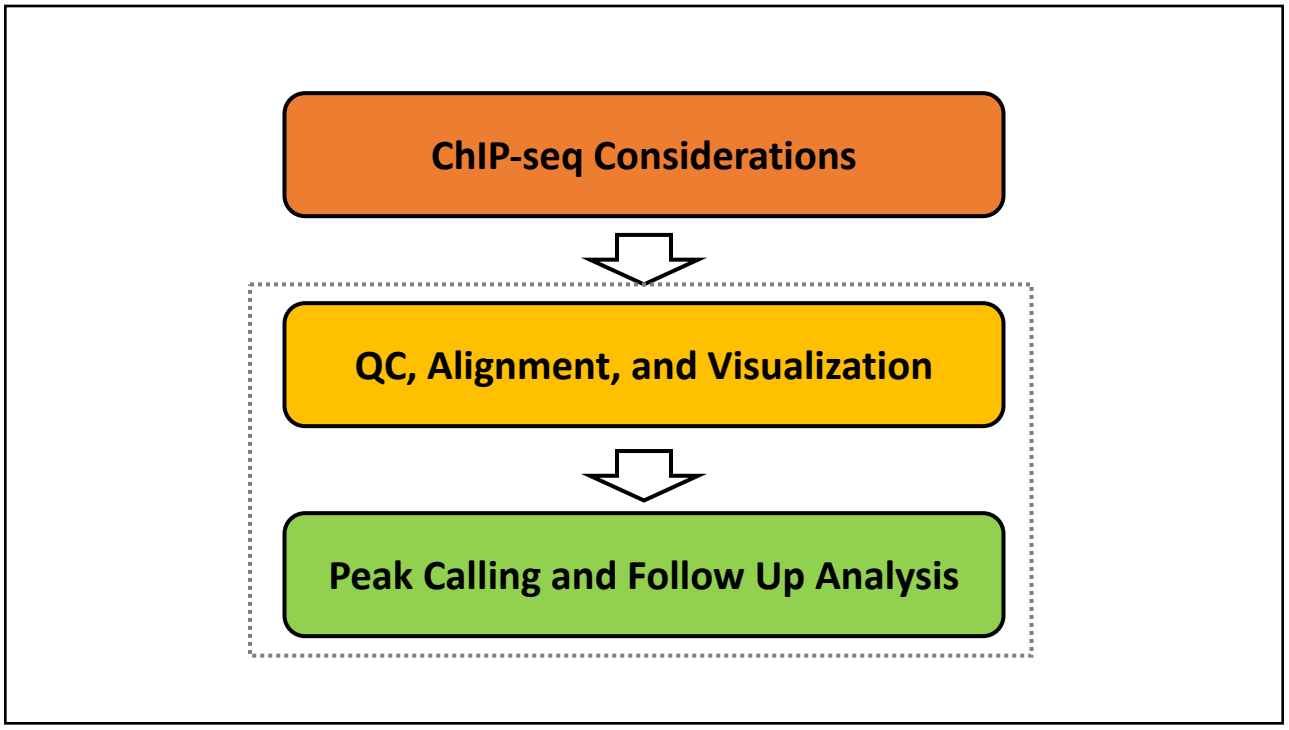
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