

Video 3:
Evaluating the quality of
your ChIP-seq data

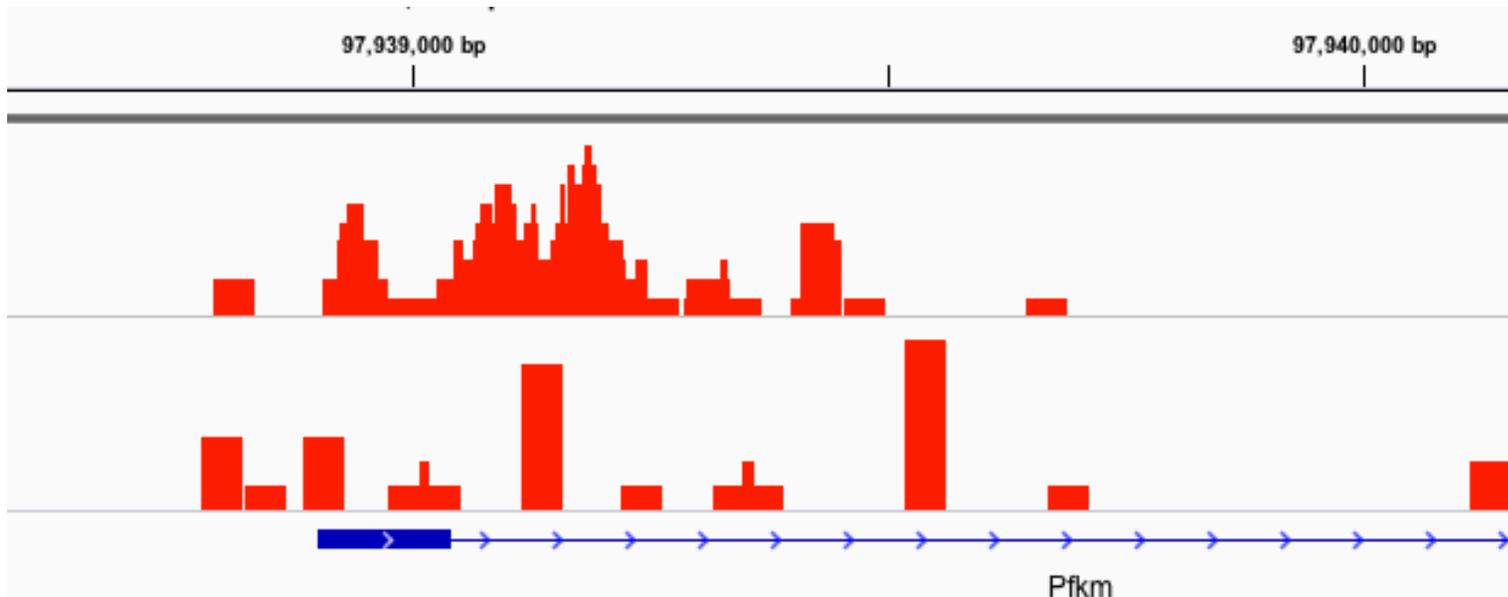
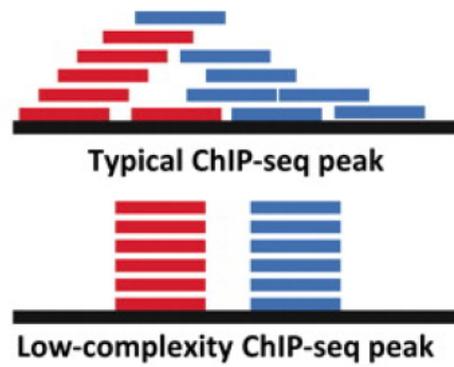
How to evaluate a ChIP-seq run?

- Key Quality Control Steps:
 - Complexity
 - Control sample
 - Measurement of global ChIP enrichment
 - Cross-correlation

Resource

**ChIP-seq guidelines and practices of the ENCODE
and modENCODE consortia**

Complexity



Complexity

- Non-redundant fraction

$$\text{NRF} = \frac{\# \text{unique start positions of uniquely mappable reads}}{\# \text{uniquely mappable reads}}$$

- Decreases with sequencing depth
- Recommend > 0.8 for 10 million uniquely mapped reads

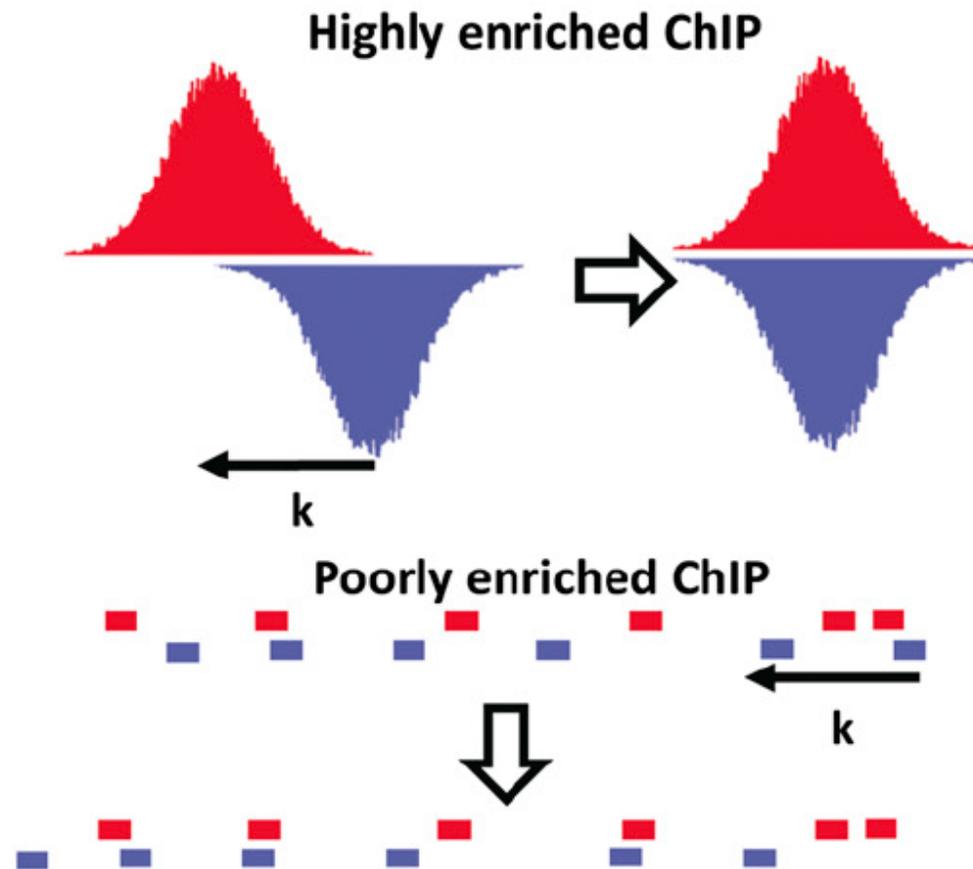
Control Sample

- Two types of control sample:
 - “Input” DNA
 - Sonicated DNA fragment w/o ChIP
 - A “mock” ChIP
 - ChIP using non-specific IgG
 - More closely mimics ChIP experiment
 - Must recover enough DNA to gain complexity of the library

Measurement of global ChIP enrichment

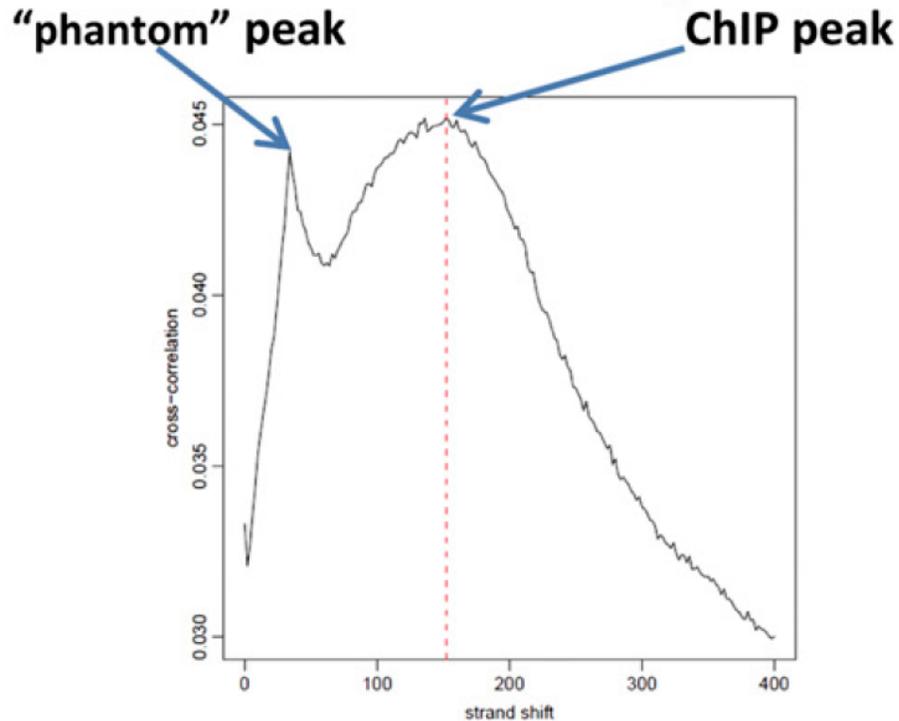
- A useful and simple first-cut metric for the success of the immunoprecipitation
- FRiP (fraction of reads in peaks)
 - Calculates the fraction of all mapped reads that fall into peak regions identified by a peak-calling algorithm.
- 1% threshold is a general guideline
- FRiP is very useful for comparing results obtained
 - with the same antibody across cell lines
 - with different antibodies against the same factor
- Affected by algorithm used for peak-calling

Cross-correlation analysis



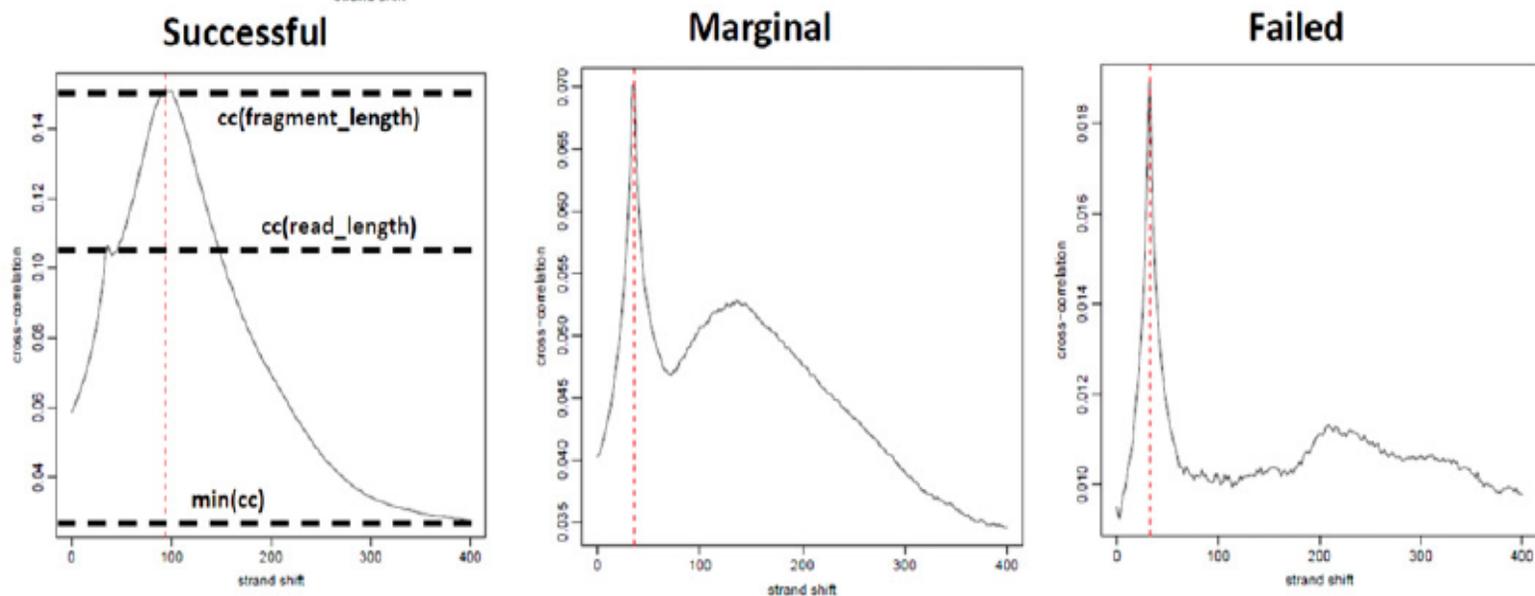
Cross-correlation analysis

- Sequence tag density accumulates on “+” and “-” strands centered around the binding site.
- It is computed as the Pearson correlation between the Crick strand and the Watson strand, after shifting Watson by k base pairs.



Cross-correlation analysis

- (Normalized Strand Coefficient) NSC and (Relative Strand Ratio) RSC are strong metrics for assessing signal-to-noise ratios in a ChIP-seq experiment.
- Consider re-do ChIP if NSC < 1.05 and RSC < 0.8



$$NSC = \frac{cc(fragment\ length)}{min(cc)}$$

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