

ChIP-Seq Analysis

Simon Andrews

simon.andrews@babraham.ac.uk

[@simon_andrews](#)

v2018-03-2

What this course covers

- The theory of ChIP-Seq
- ChIP-Seq library properties
- Sequencing, Data processing and QC
- Data visualisation and exploration
- Types of analysis
 - Peak Calling
 - Differential Binding

What is ChIP-Seq

ChIP-Seq is a technology which uses high-throughput sequencing to infer the positions of any mark associated with DNA which can be captured by an antibody.

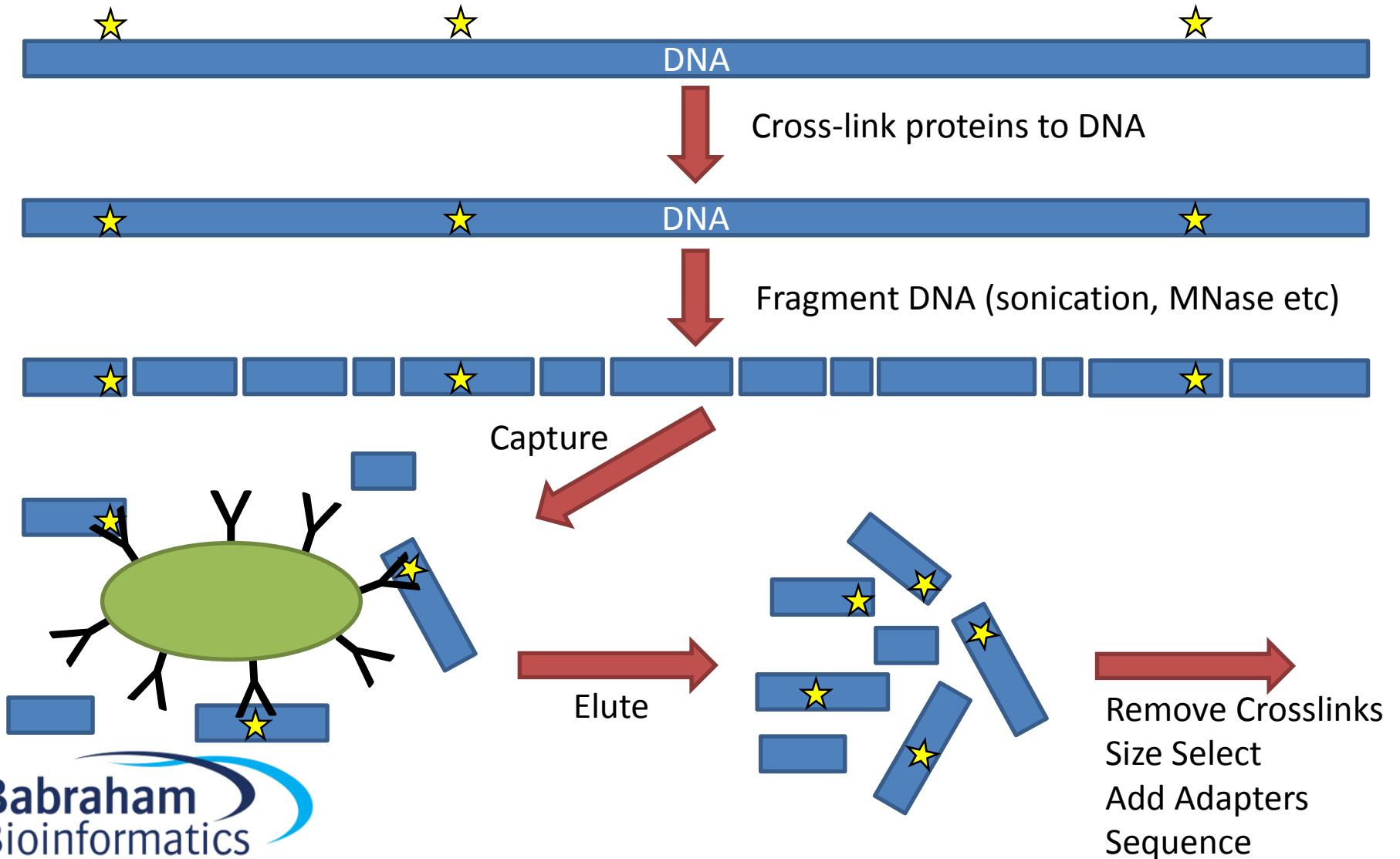
Types of antibody

- Transcription factors / repressors
 - nanog, CTCF
- Histones and histone modifications
 - H3, H3K4me3
- DNA modifications
 - Methyl-Cytosine, Formyl cytosine
- Chromatin remodelling proteins
 - BMI1, EZH2
- Transcription machinery
 - Pol2

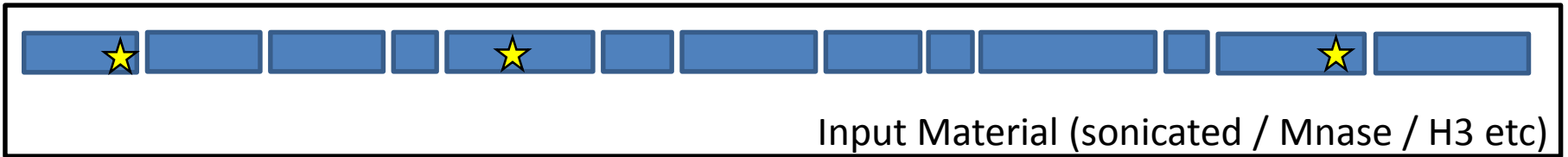
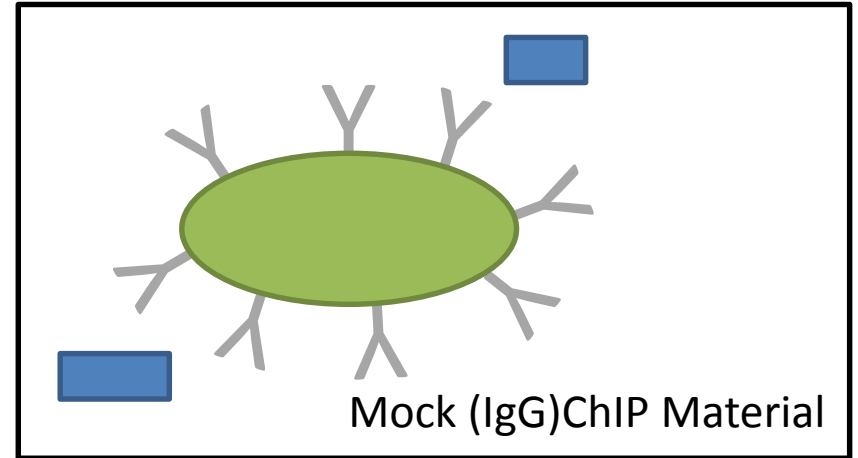
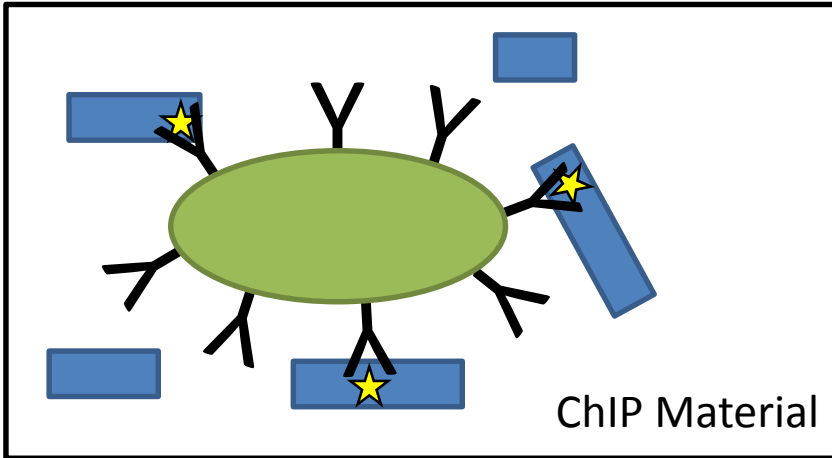
Related Techniques

- ATAC-Seq
 - Uses transposases to digest exposed DNA to enrich for accessible DNA.
- DamID/DamIP
 - Fuses a methyltransferase to a protein then measures methyl-Adenine by bisulphite seq (DamID) or mA ChIP (DamIP)

How Does ChIP-Seq work



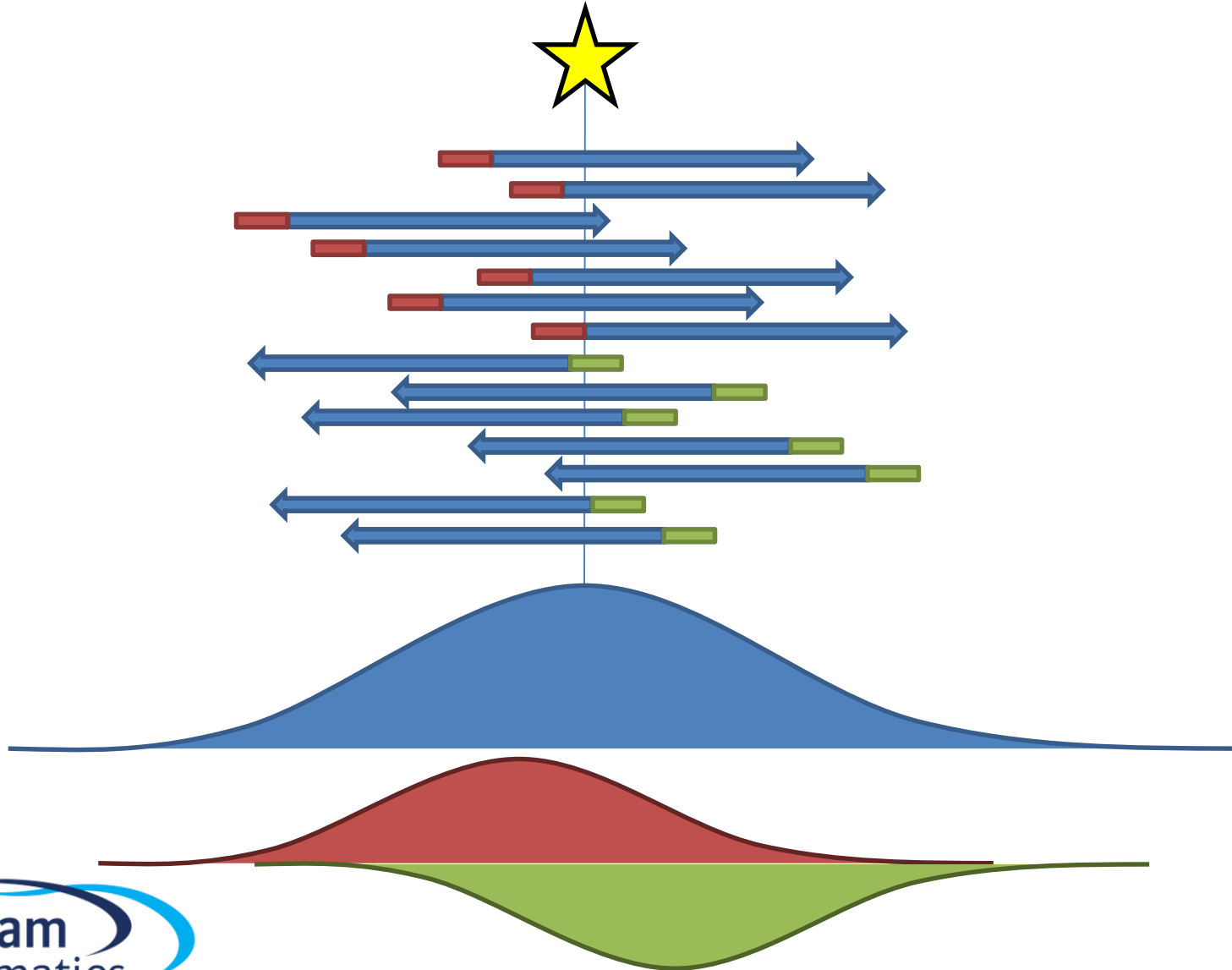
What can you sequence?



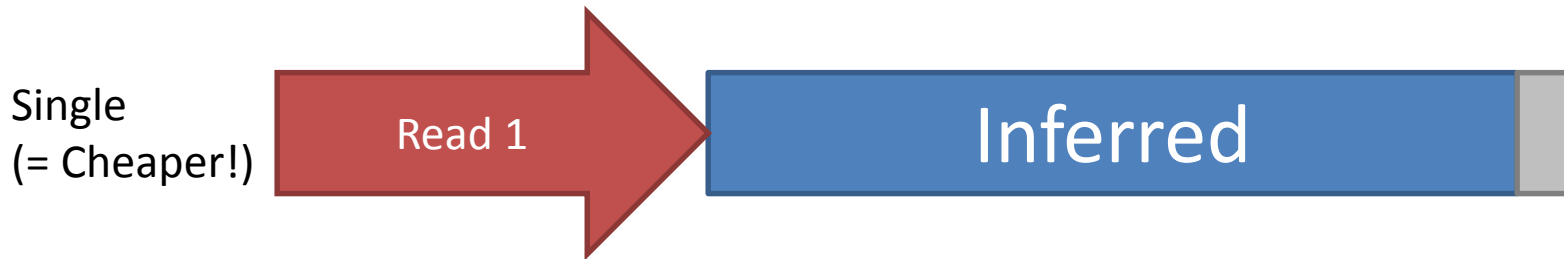
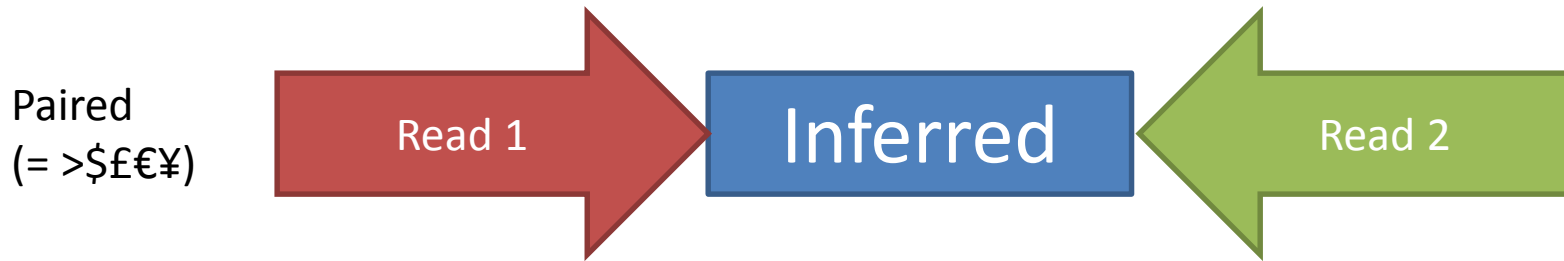
Sequencing for ChIP



What you end up with

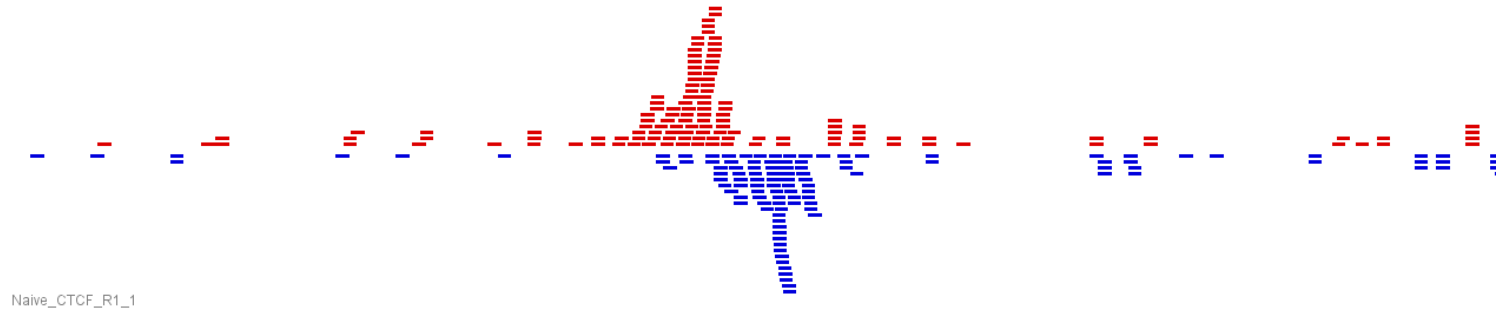


Single End vs Paired End

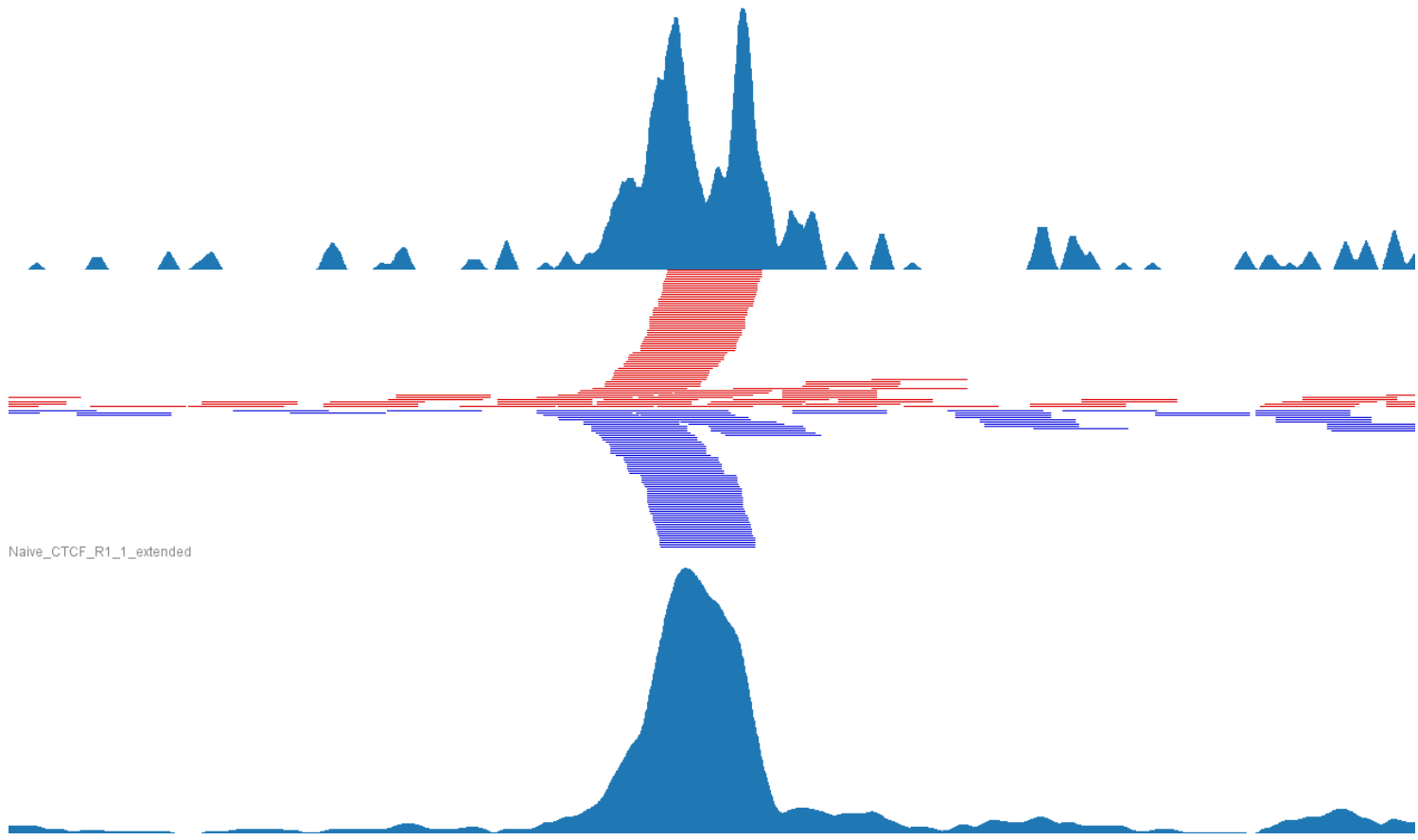


What you end up with

Original
40bp
Reads

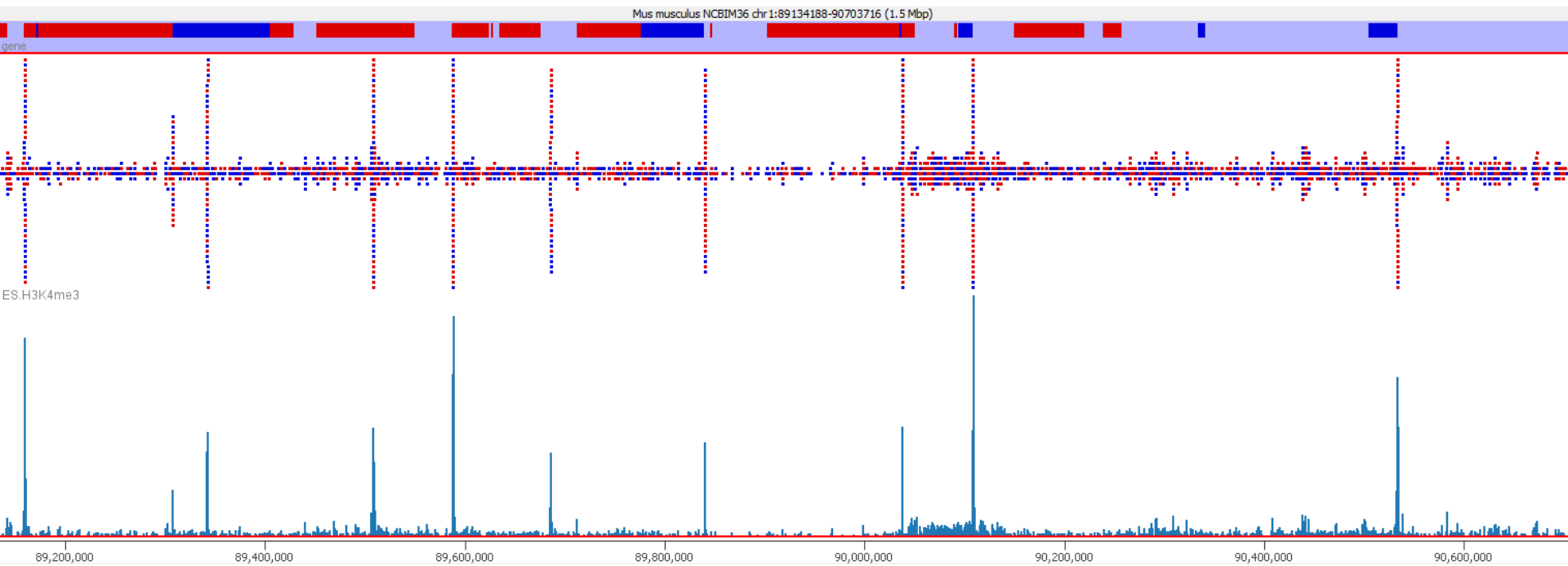


Extended
by
250bp



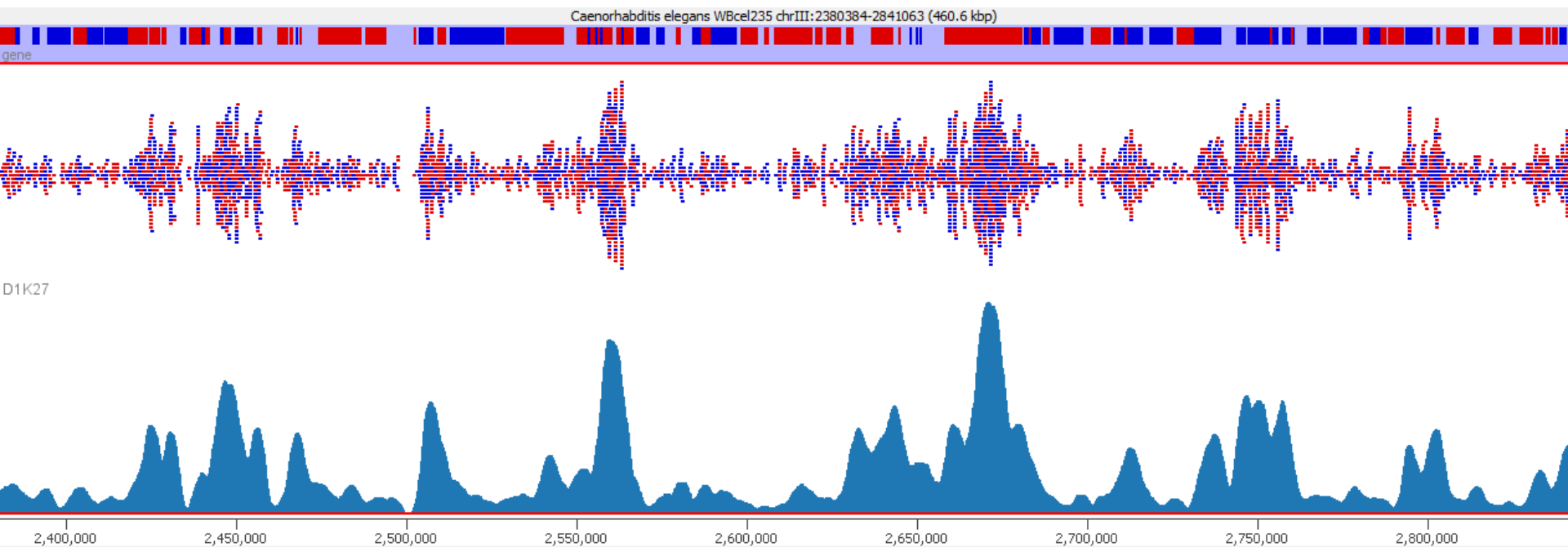
Types of Enrichment

- Single points (typical TF, some histone marks)



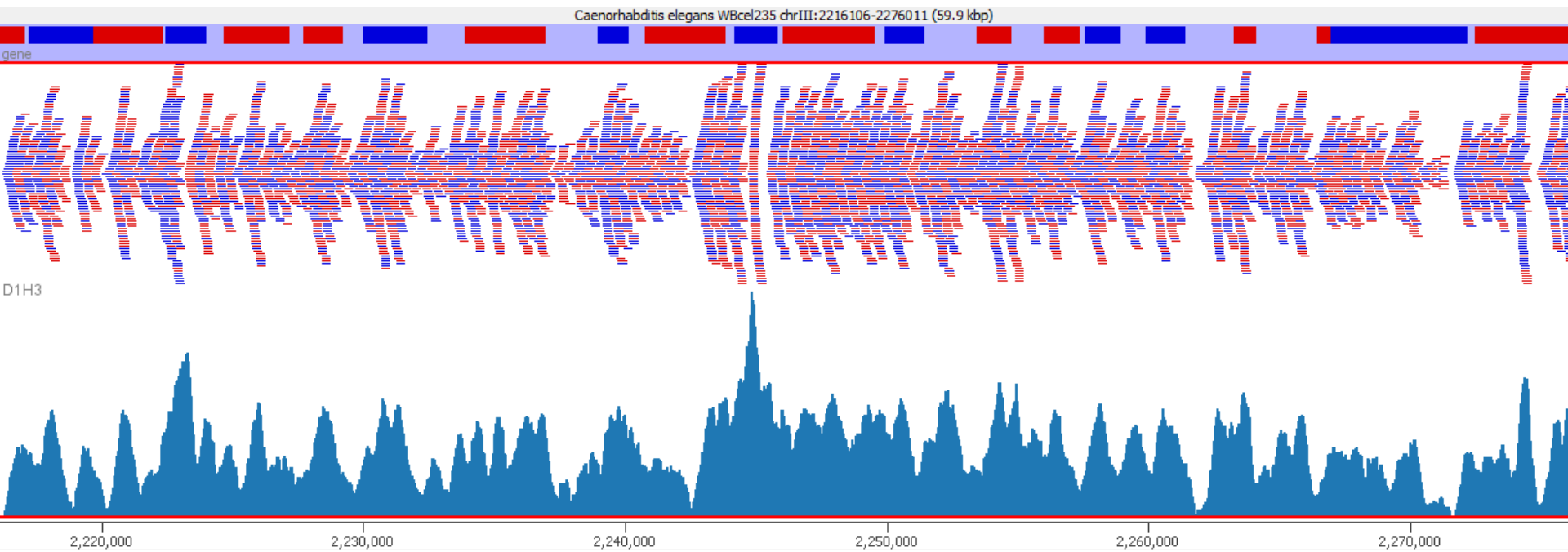
Types of Enrichment

- Broad Regions (some histone marks, PolII)



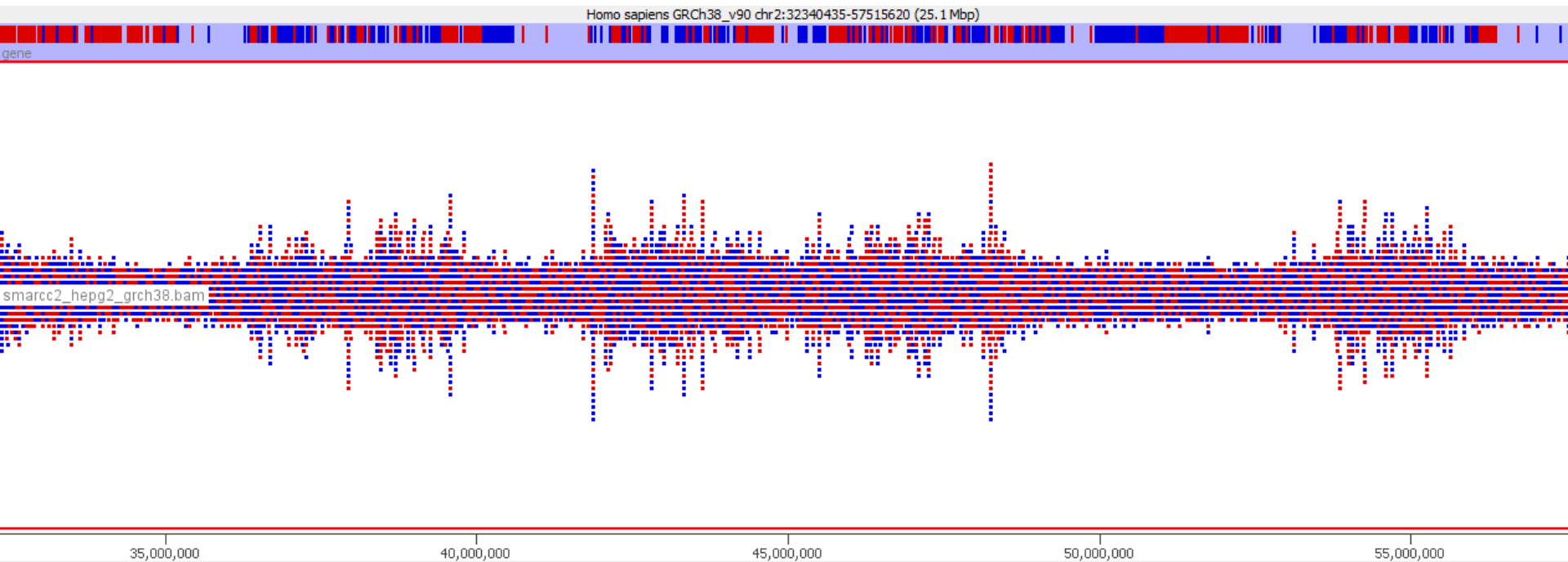
Types of Enrichment

- Virtually everywhere (h3)



Types of Enrichment

- Widely distributed (MeDIP etc - also GC!)

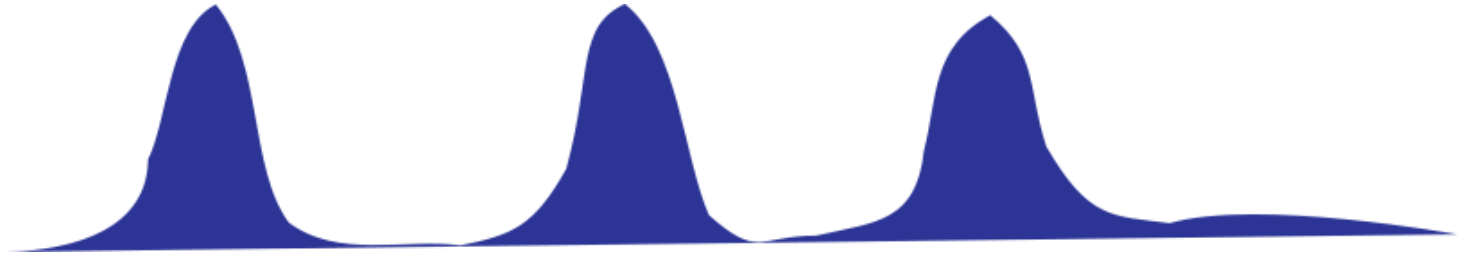


What are you actually measuring?

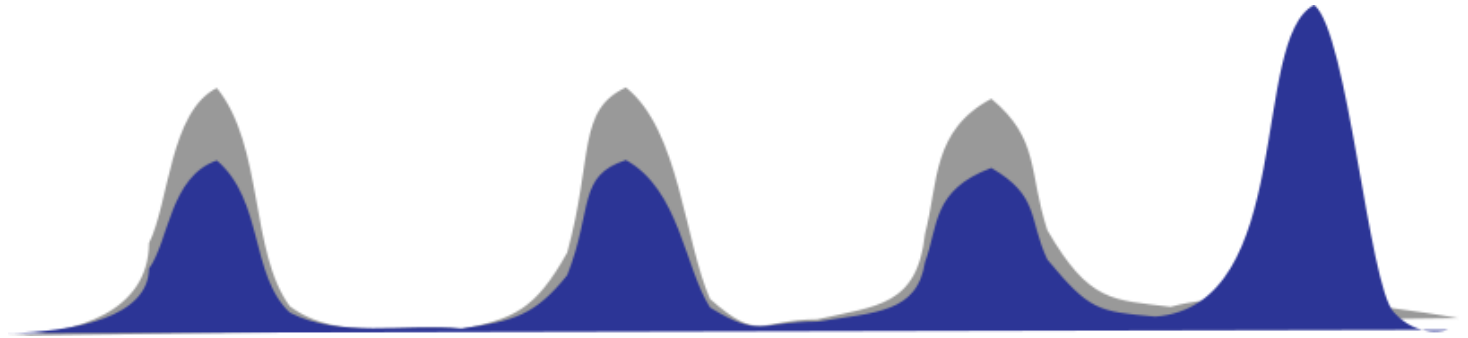
- ChIP Seq measures **RELATIVE** enrichment
 - Region A has twice as much signal as Region B
- Without some external calibration, **NOTHING** in ChIP-Seq gives an **ABSOLUTE** measure.

What can affect enrichment?

Starting



More Sites



Poorer Signal



What sort of questions can you answer?

- Where is this mark present?
 - General - it's in promoters, gene bodies etc
 - Specific - it's at these loci
- How does this mark change when I do XXX?
 - Categorical: A peak disappears
 - Quantitative: The enrichment of a locus changes