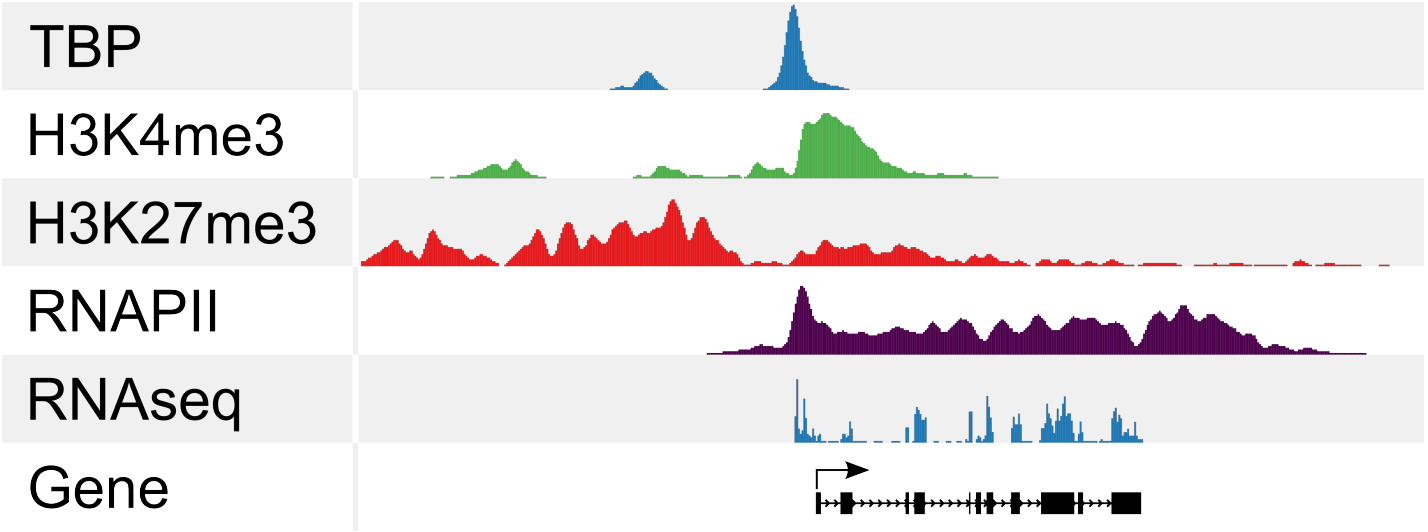
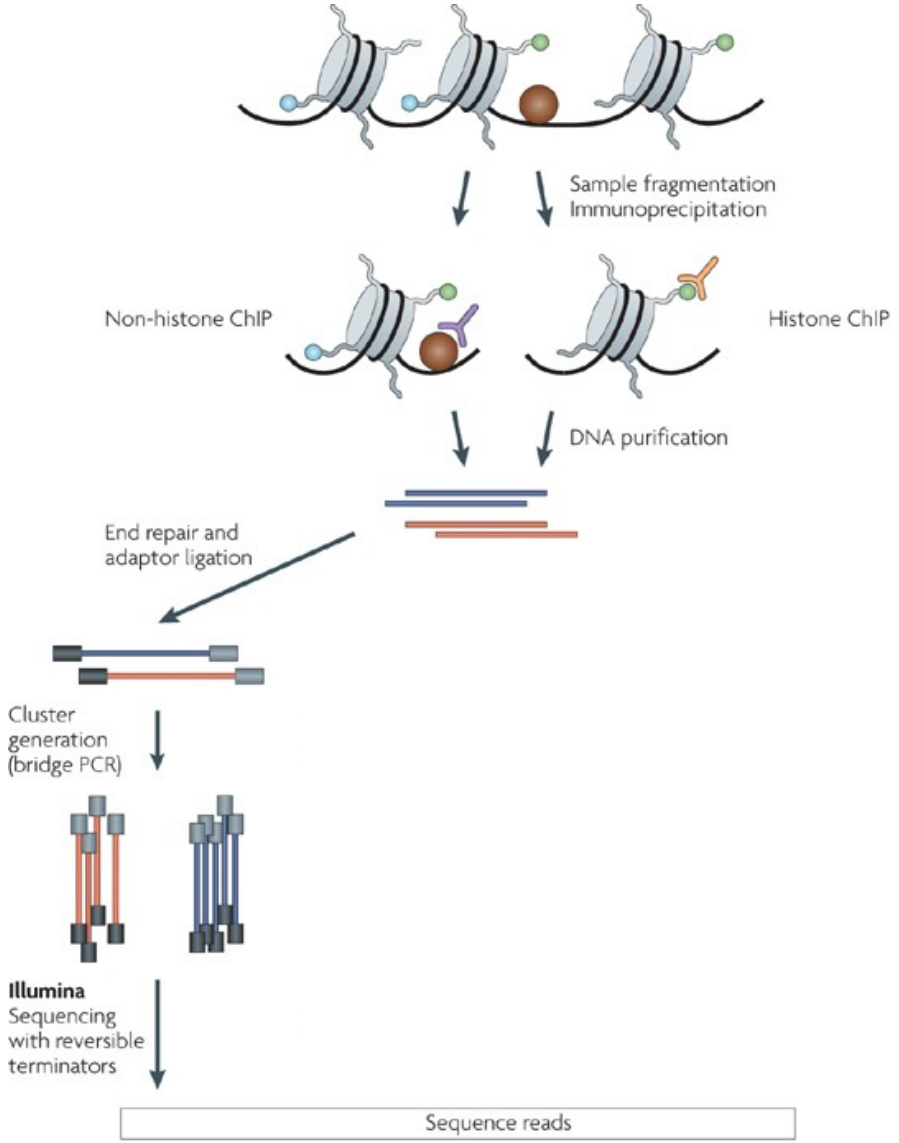


Introduction to ChIP-sequencing

Simon van Heeringen
November 4, 2014

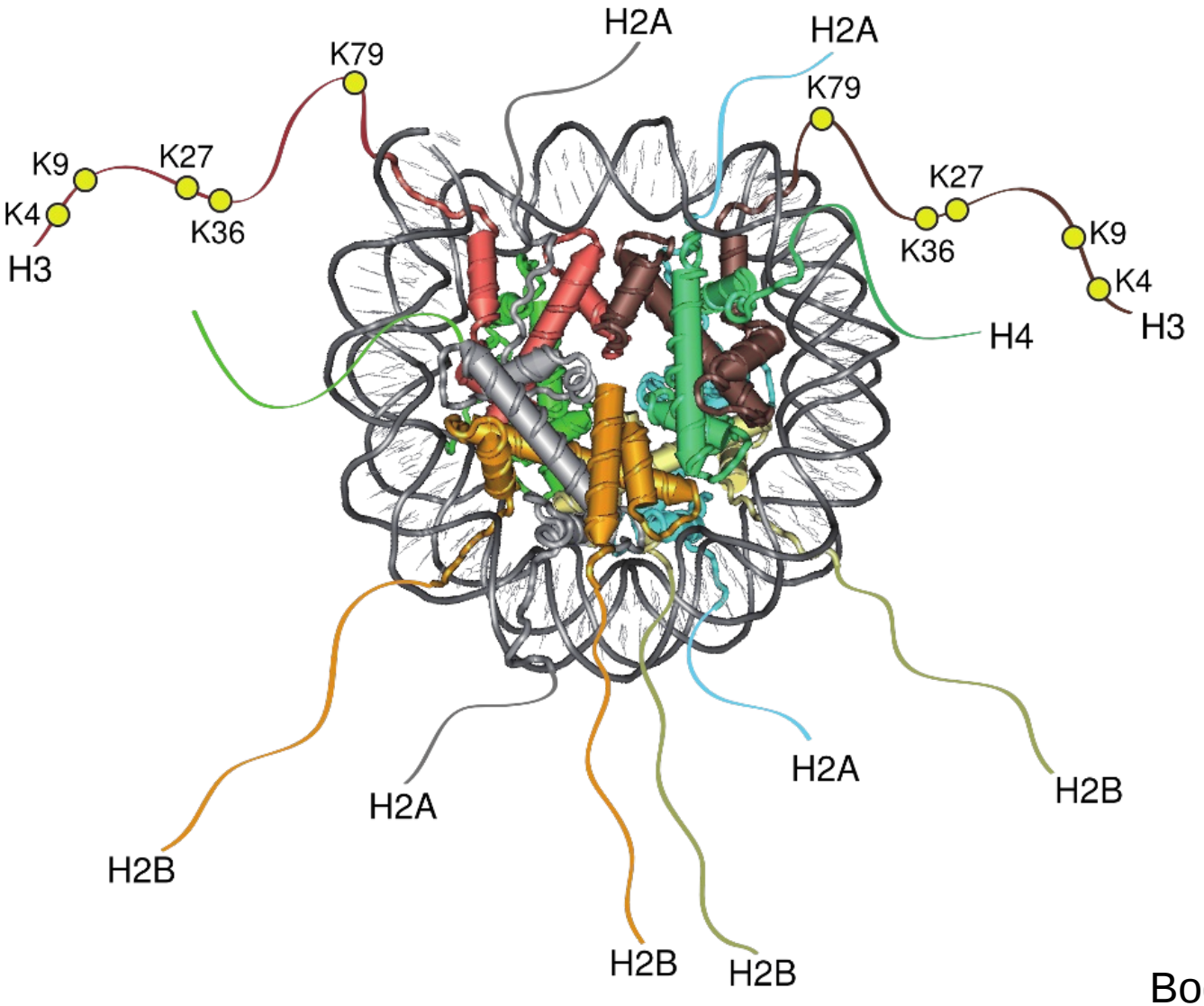
A basic ChIP-seq experiment



- Cross-link protein and DNA (formaldehyde)
- Fragmentation (sonication)
- IP with antibody
- Reverse crosslinks
- Amplification, library preparation
- Sequencing

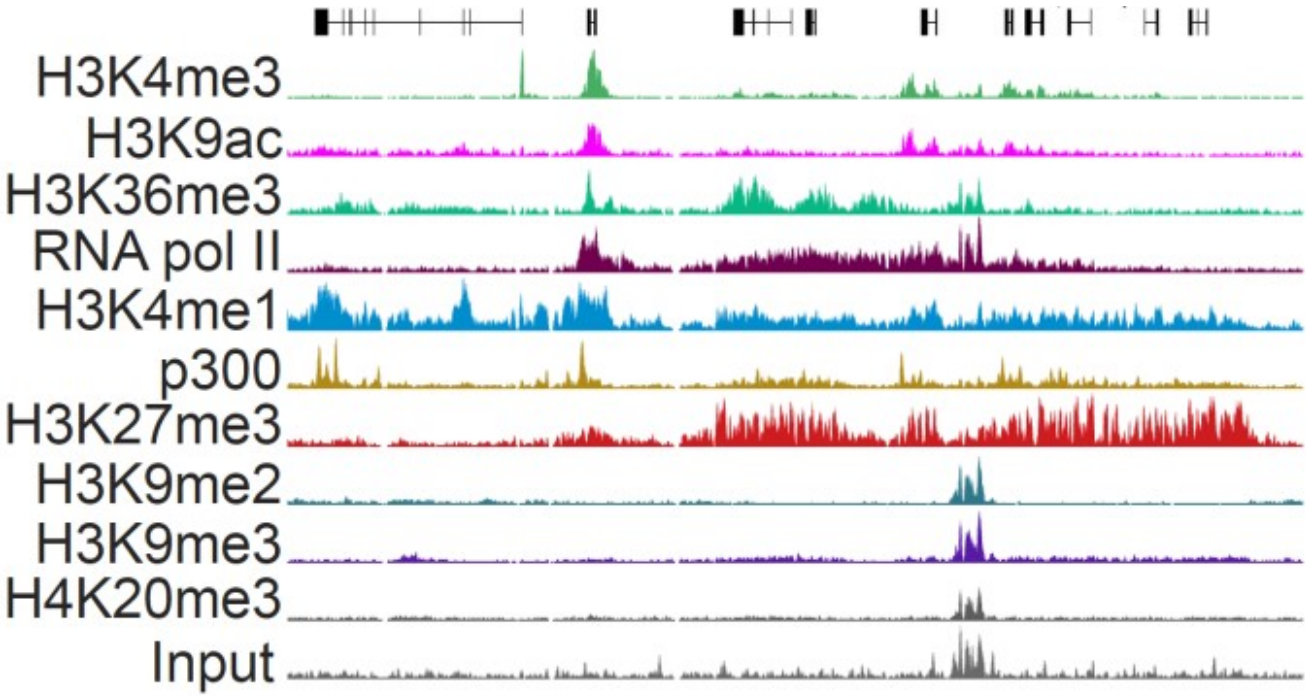
Adapted from Park, 2009

Histone modifications

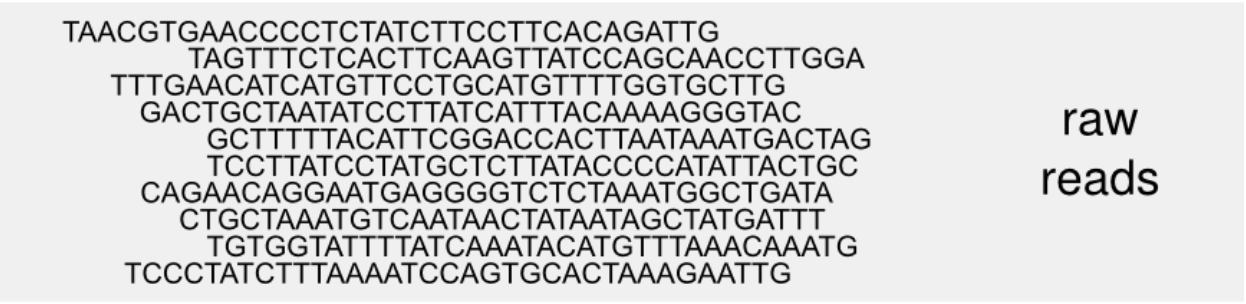
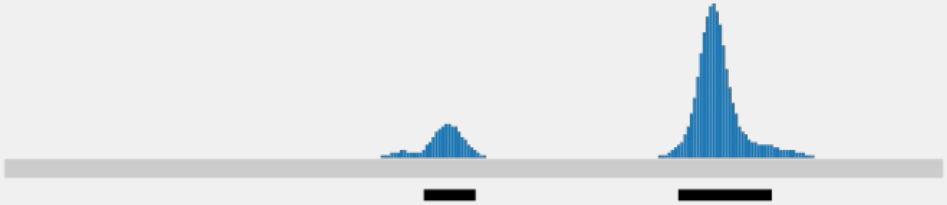


Bogdanovic, 2012

Epigenome


H3K4me3
H3K9ac
H3K36me3
RNA pol II
H3K4me1
p300
H3K27me3
H3K9me2
H3K9me3
H4K20me3
Input

ChIP-seq workflow

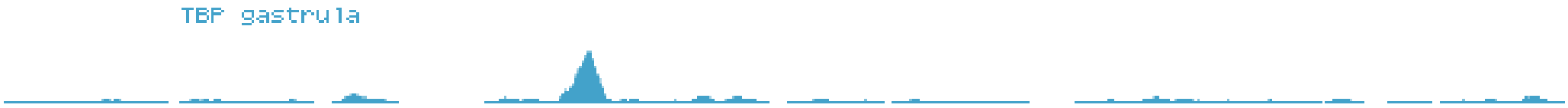
	Data type	File formats
	raw reads	FASTQ SAM / BAM
	aligned data	BED SAM / BAM
	visualization, peak calling	BED WIG

Basic downstream analysis

- Visualization
  - Genome Browser
- Identifying enriched regions (peaks)
- Functional analysis
- Transcription factor motifs

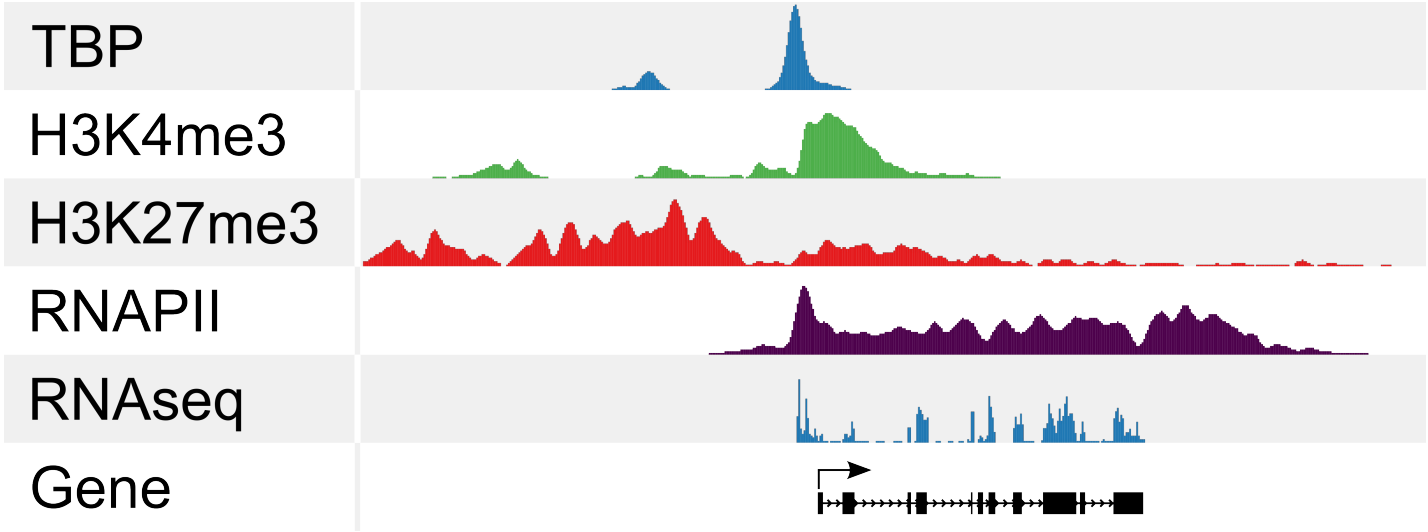
Peak calling

- Sounds easy, doesn't it?

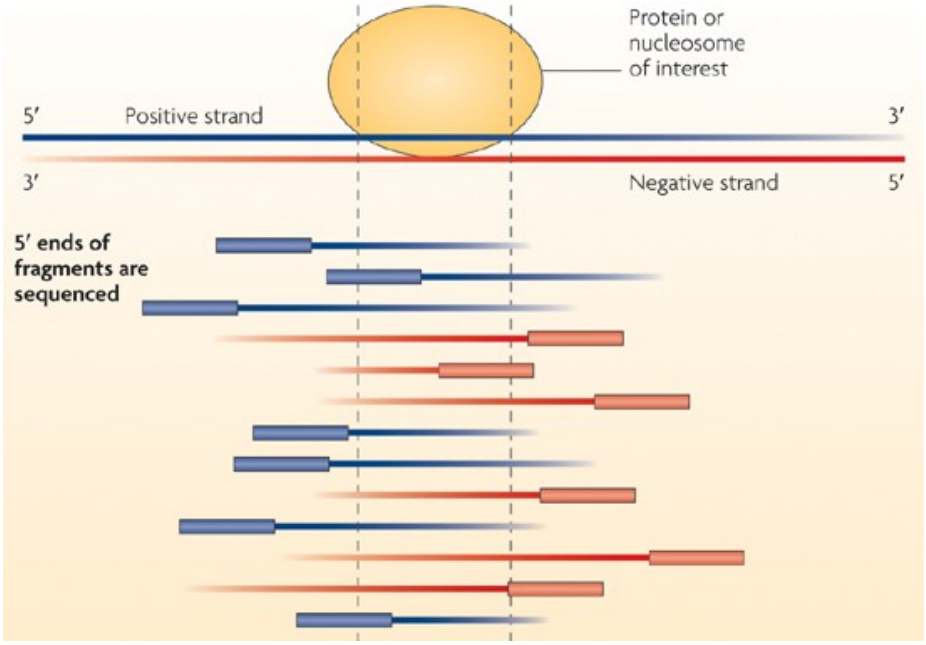


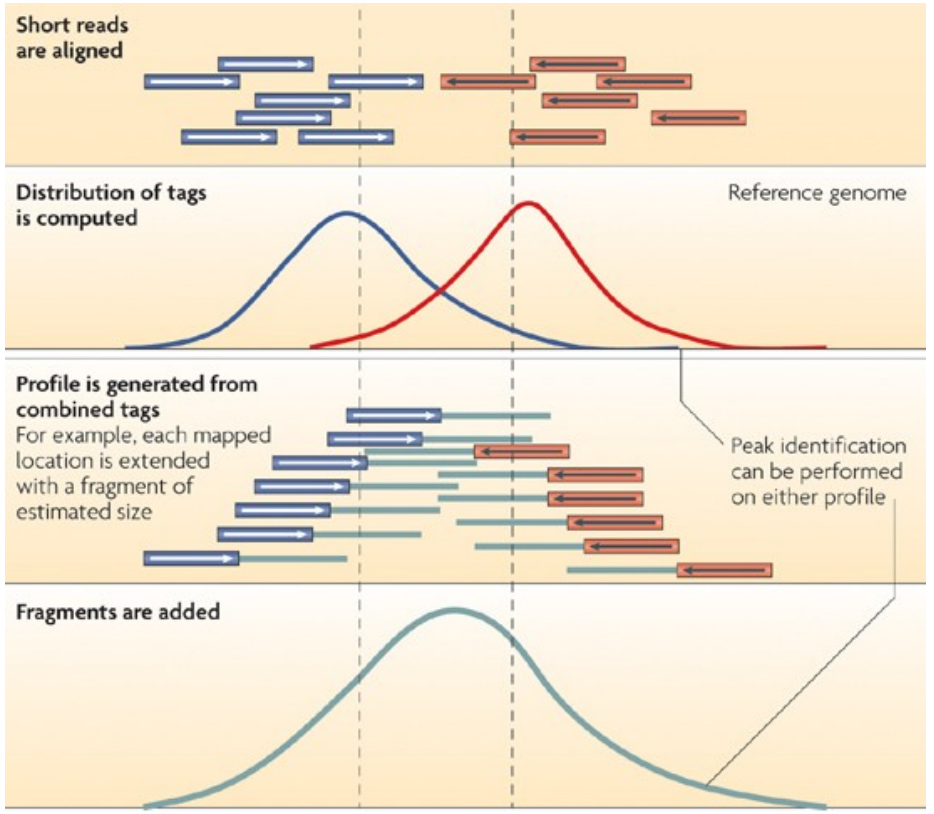
Peak!

Peak calling



Peak calling





Park, 2009

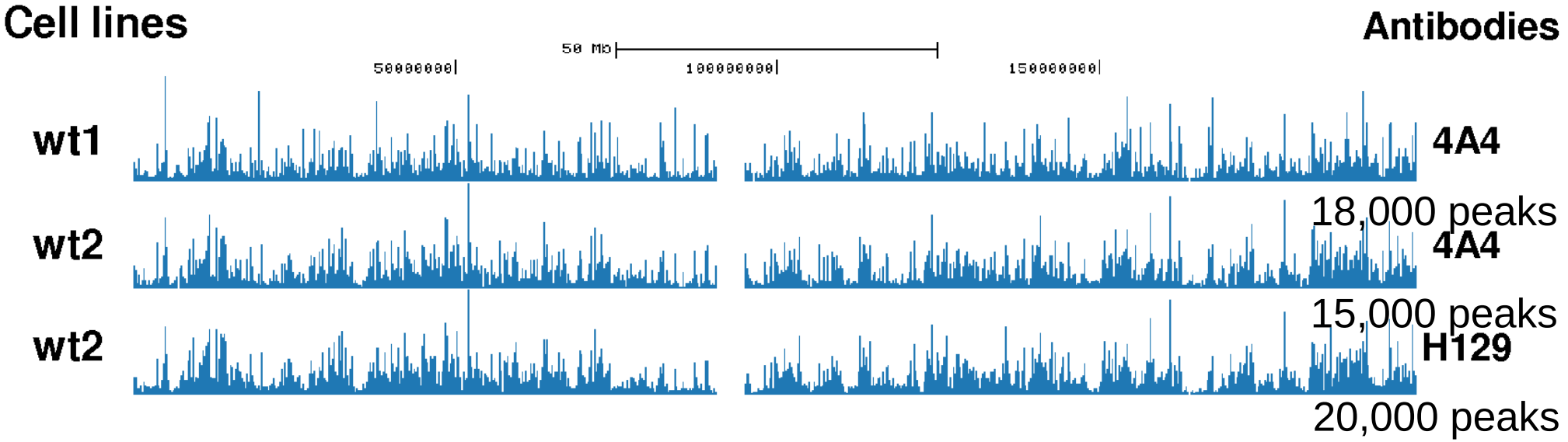
ENCODE guidelines

- Antibody specificity
- At least 2 biological replicates
- Control per cell line / stage / condition
  - IgG, Input DNA
- Read depth
  - “Point” versus “broad”

Landt, 2012

Experimental design: Replicates

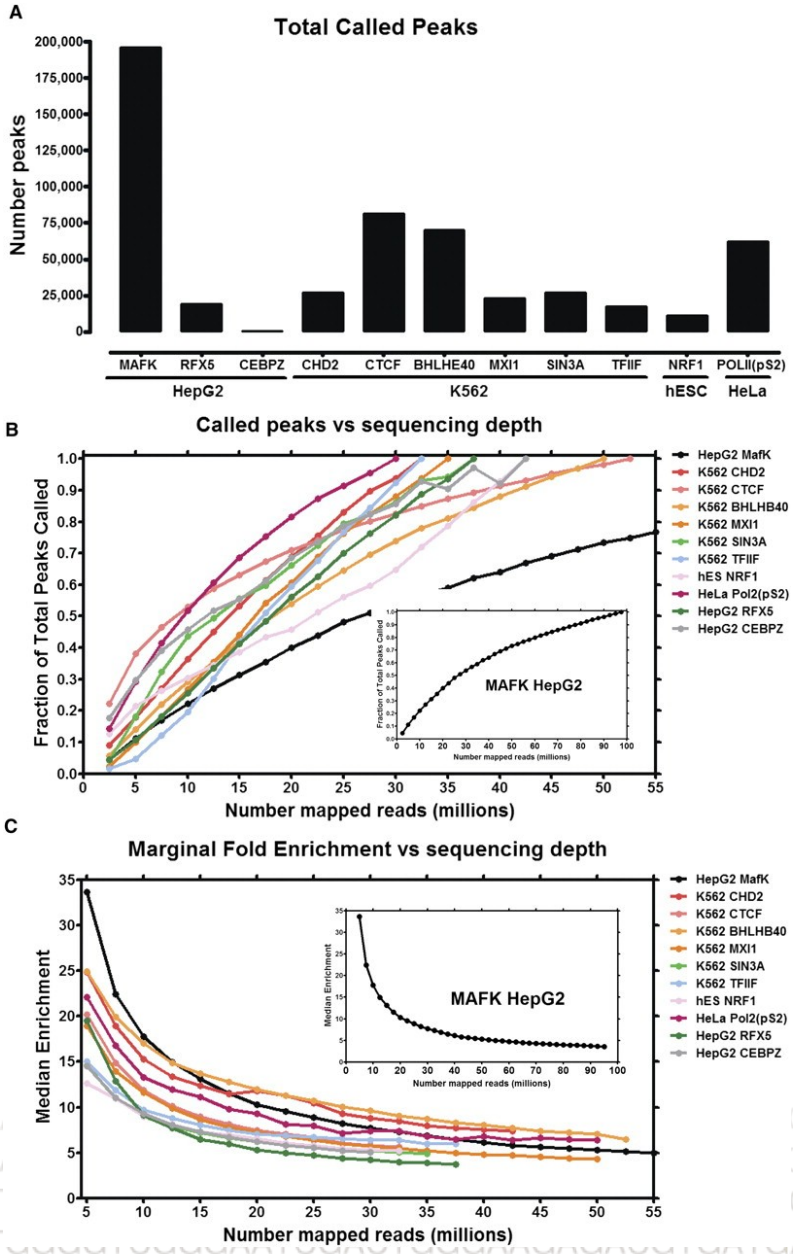
- ChIP-seq of p63 in primary human keratinocytes



- ~20,000 peaks, ~11,000 shared
- 99% of the peaks were present in third replicate!

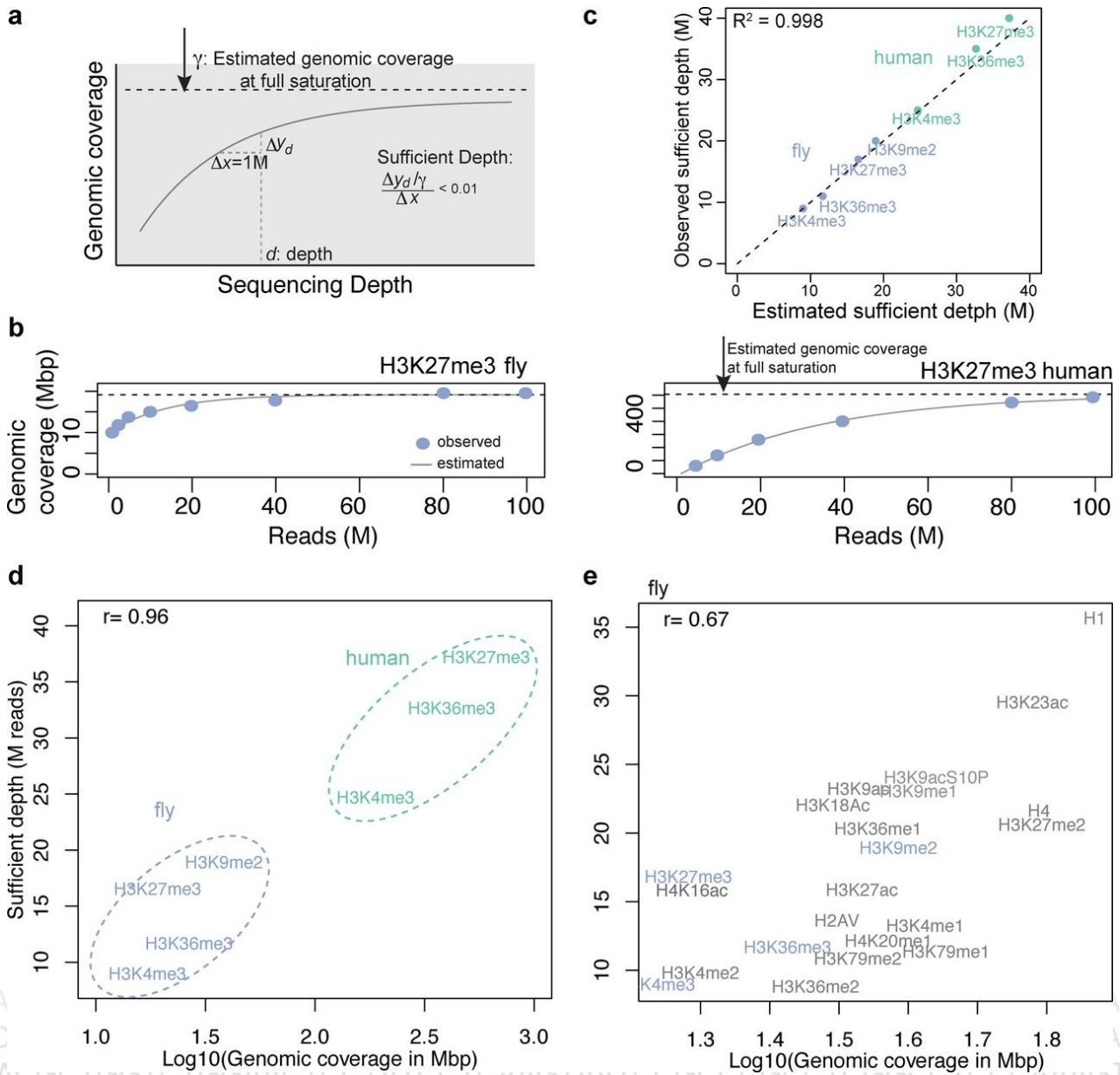
Kouwenhoven *et al.* 2010

Read depth, saturation



Landt, 2012

Read depth, saturation



Jung, 2014

Server IP addresses

- 54.80.155.122
- 54.162.253.10
- 54.167.247.172
- 54.205.72.202
- 54.82.190.179
- 54.162.100.112
- 54.242.87.148