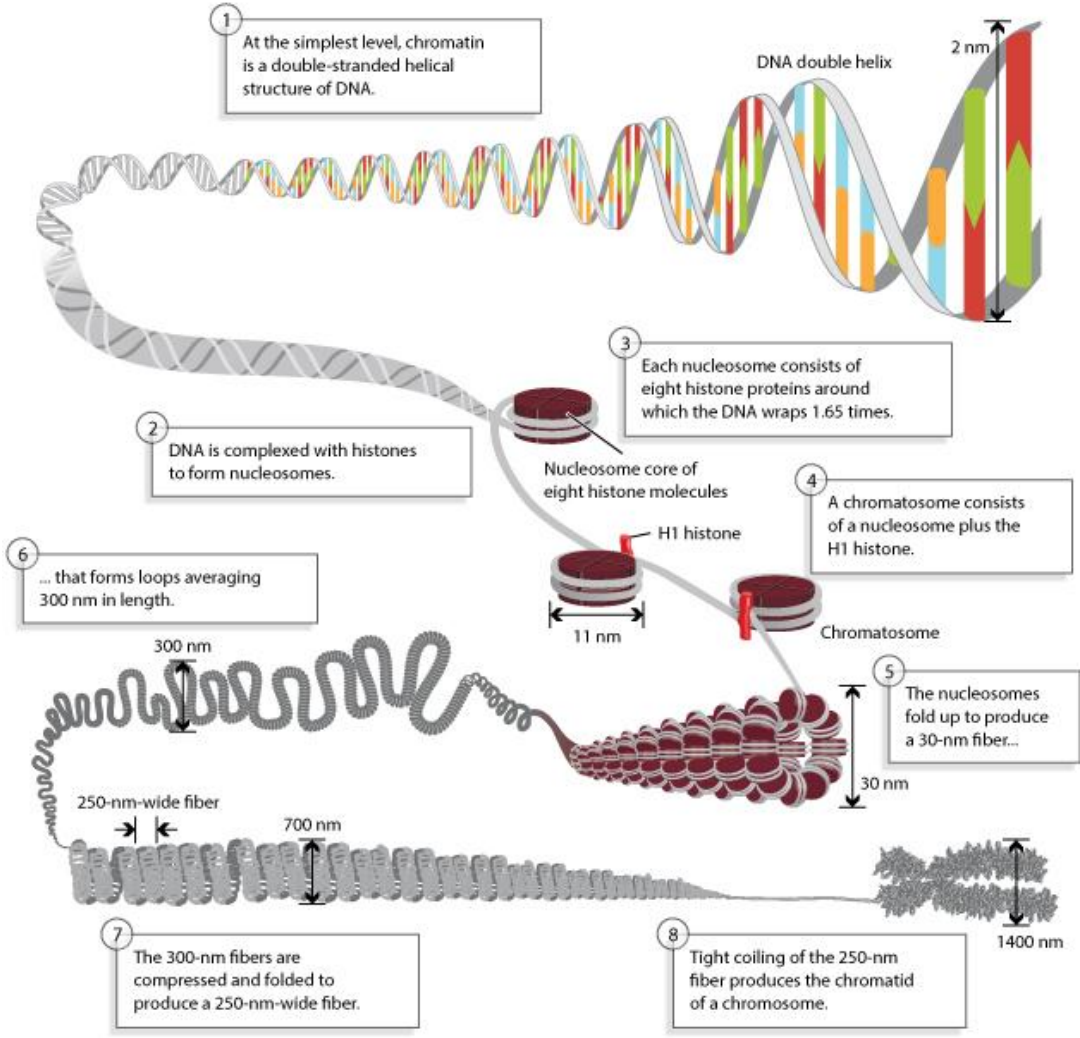
Introduction to ChIP-seq

Overview, alignment, peaks, and motifs

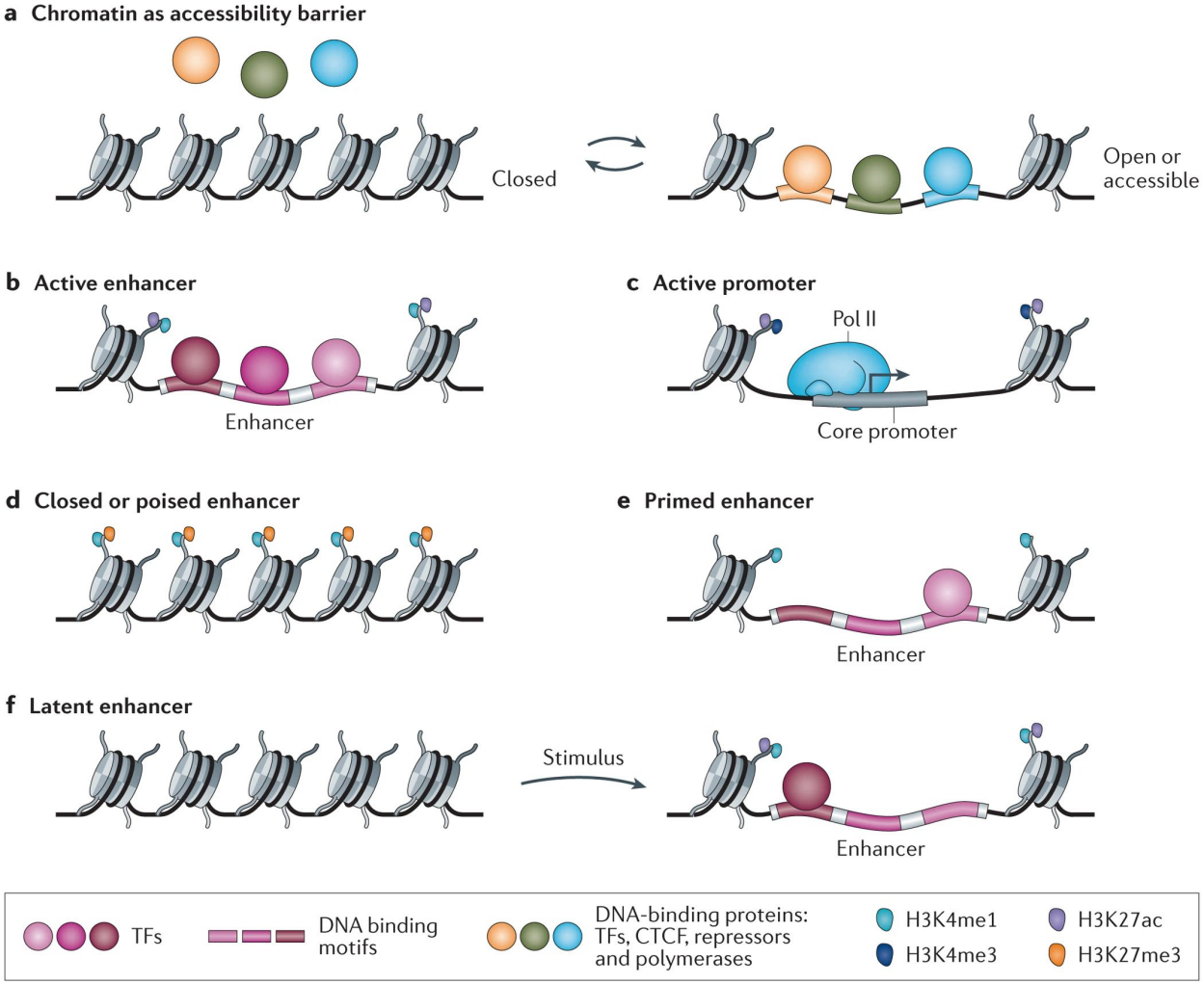
DNA hierarchy

- Multiple levels of organization for DNA, histones, and chromatin
- For ChIP-seq, our interest is in levels 2 and 3
- Related assays probe for chromatin accessibility



Gene regulation

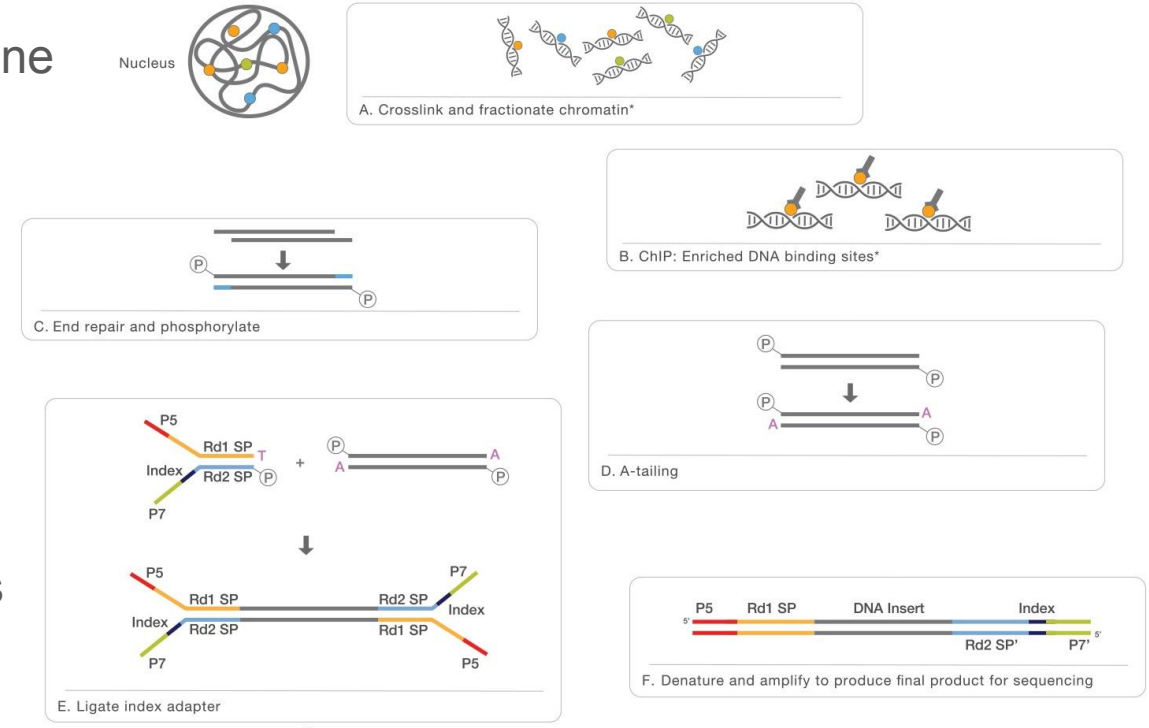
- Chromatin elements play a vital role in regulating gene expression



ChIP-seq protocol

- An assay for studying gene regulation
  - Promoters
  - Enhancers
  - Repressors

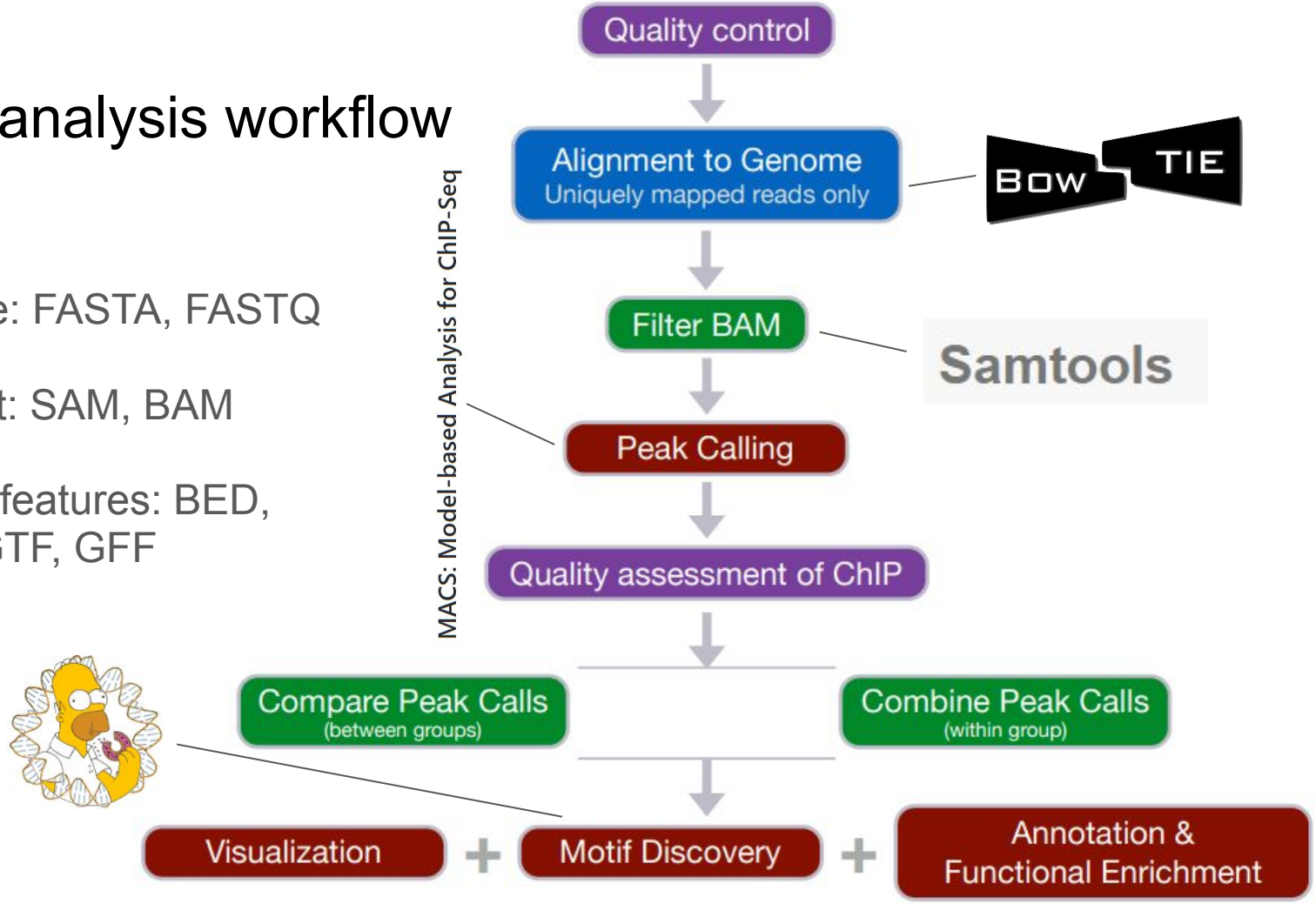
- Profiling protein binding (ChIP) of DNA (seq)
  - Transcription facotrs
  - Histone modifiers



ChIP-seq analysis workflow

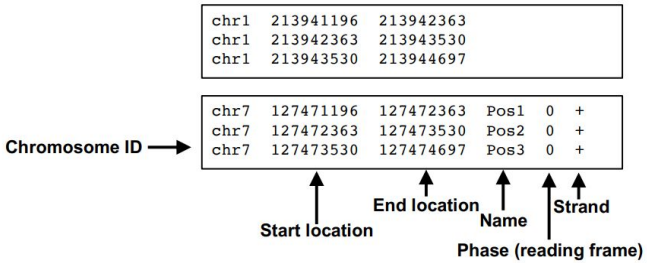
Data types

1. Sequence: FASTA, FASTQ
2. Alignment: SAM, BAM
3. Genomic features: BED, Wiggle, GTF, GFF



BED file format

1. chrom: name of the chromosome
2. chromStart: starting position of the feature (0-base)
3. chromEnd: ending position of the feature



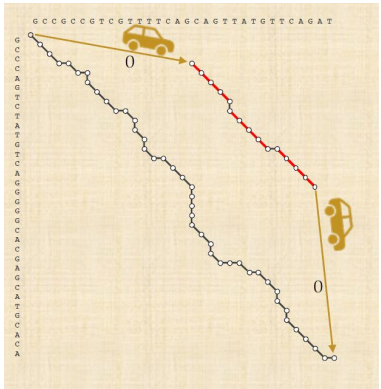
(optional columns 4-6)

4. name: name for the BED line
5. score: quality score (sometimes omitted in place of something else)
6. strand: “+” (sense), “-” (antisense), “.” (NA)

(optional columns 7-12, see UCSC Genomics Institute page)

Read alignment

- Bowtie2, Bowtie1, and bwa are all suitable methods dependent on read length
- We have to construct an index
- Can perform local or global alignment



Local Alignment

```
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
          |||| ||||||| ||||||||||||||||
     5' TACTCACGGATGAGGTACTTTAGAGGC 3'
```

Global Alignment

```
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
   ||||||||||||    |||||||  |||||||||||||| |||||||
5' ACTACTAGATT----ACGGATC--GTACTTTAGAGGCTAGCAACCA 3'
```

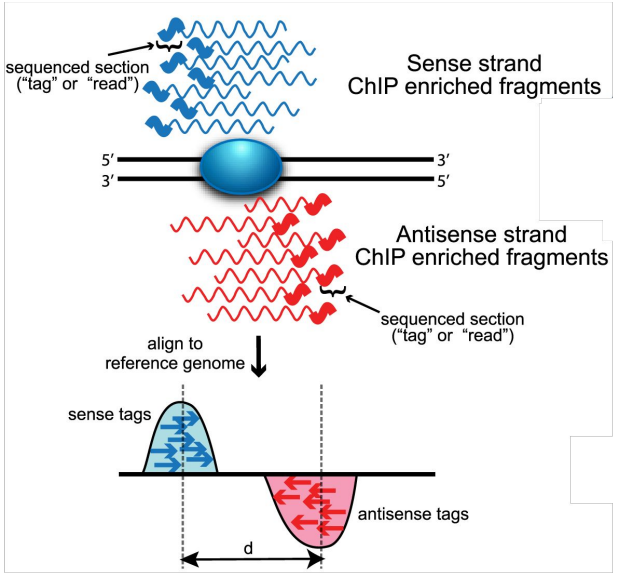
Quality control

```
[zemke@n7420 flashscratch]$ bowtie2 -q -p 4 -k 1 --local
mso_k18_chip.fastq > dmso_k18_chip.sam
42714660 reads; of these:
  42714660 (100.00%) were unpaired; of these:
    980458 (2.30%) aligned 0 times
    41734202 (97.70%) aligned exactly 1 time
    0 (0.00%) aligned >1 times
97.70% overall alignment rate
```

- Non-redundant fraction
  - How many reads were uniquely mapped?
- Fraction of reads in peaks
  - Proportion of reads in significant regions of the genome?
  - Reference peak set
- Blacklist region
  - Regions of the genome where reads should be thrown out

The ENCODE Blacklist: Identification of Problematic Regions of the Genome

Peak calling

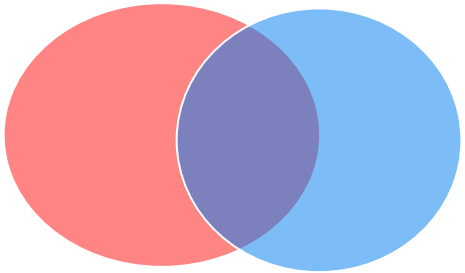


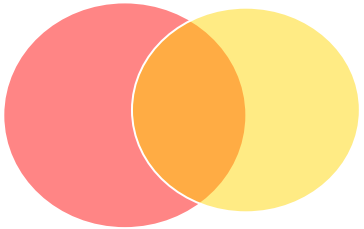
- We want to identify regions of the genome where many aligned reads are found
- MACS tests whether the background is Different from peaks using Poisson models
- Duplicates should be removed prior to peak calling otherwise there will be false positives

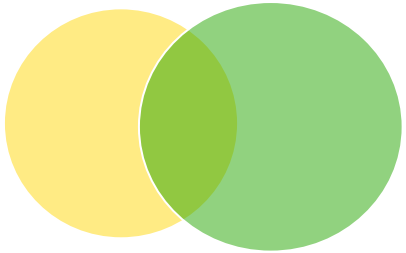
Differential peaks

Peaks that are unique to some set may be associated with specific motifs

	H3K27ac (enhancer)	H3K4me3 (repressor)
treatment	A / input	C / input
control	B / input	D / input







Motif analysis

- Peaks that are differentially expressed may have specific sequence motifs

- HOMER compares the peak set against a background set or reference genome using the binomial distribution
    - Consider the search range around peak for identifying motifs