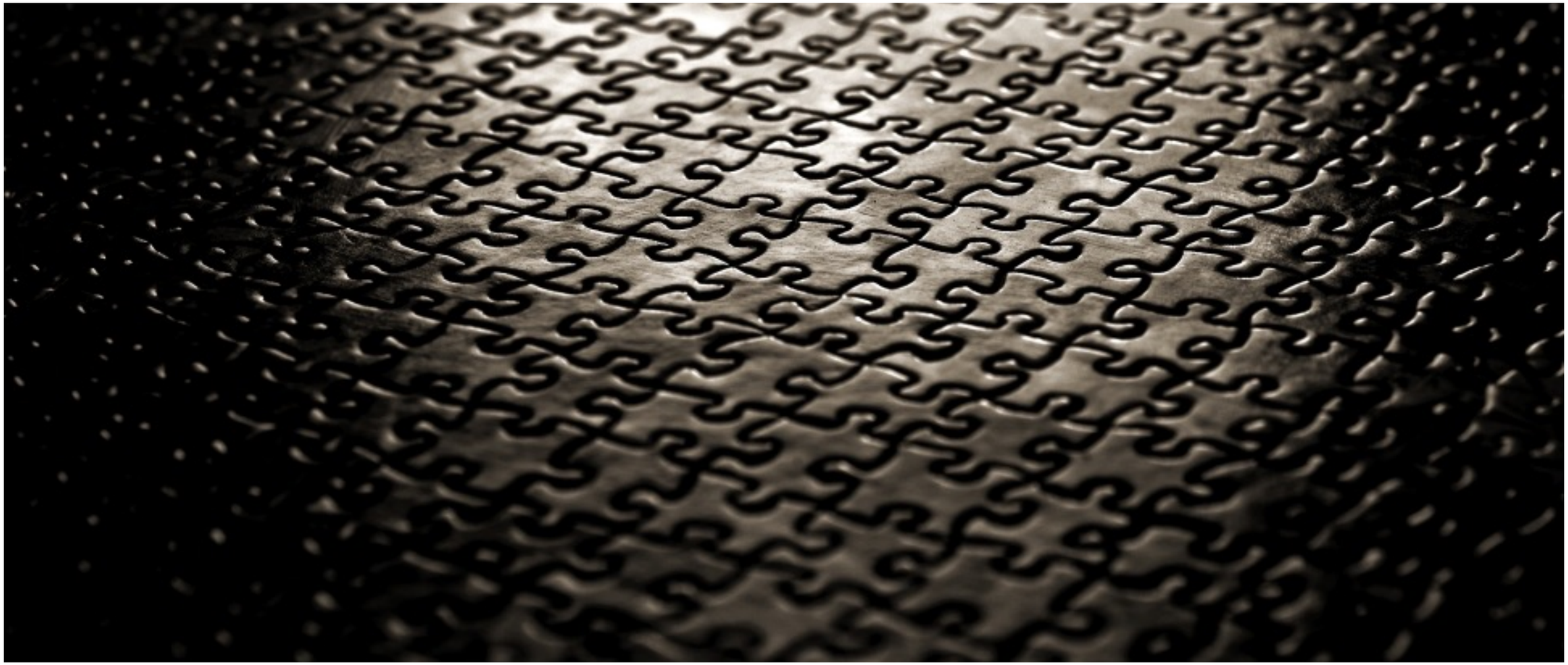


Aligning reads: tools and theory

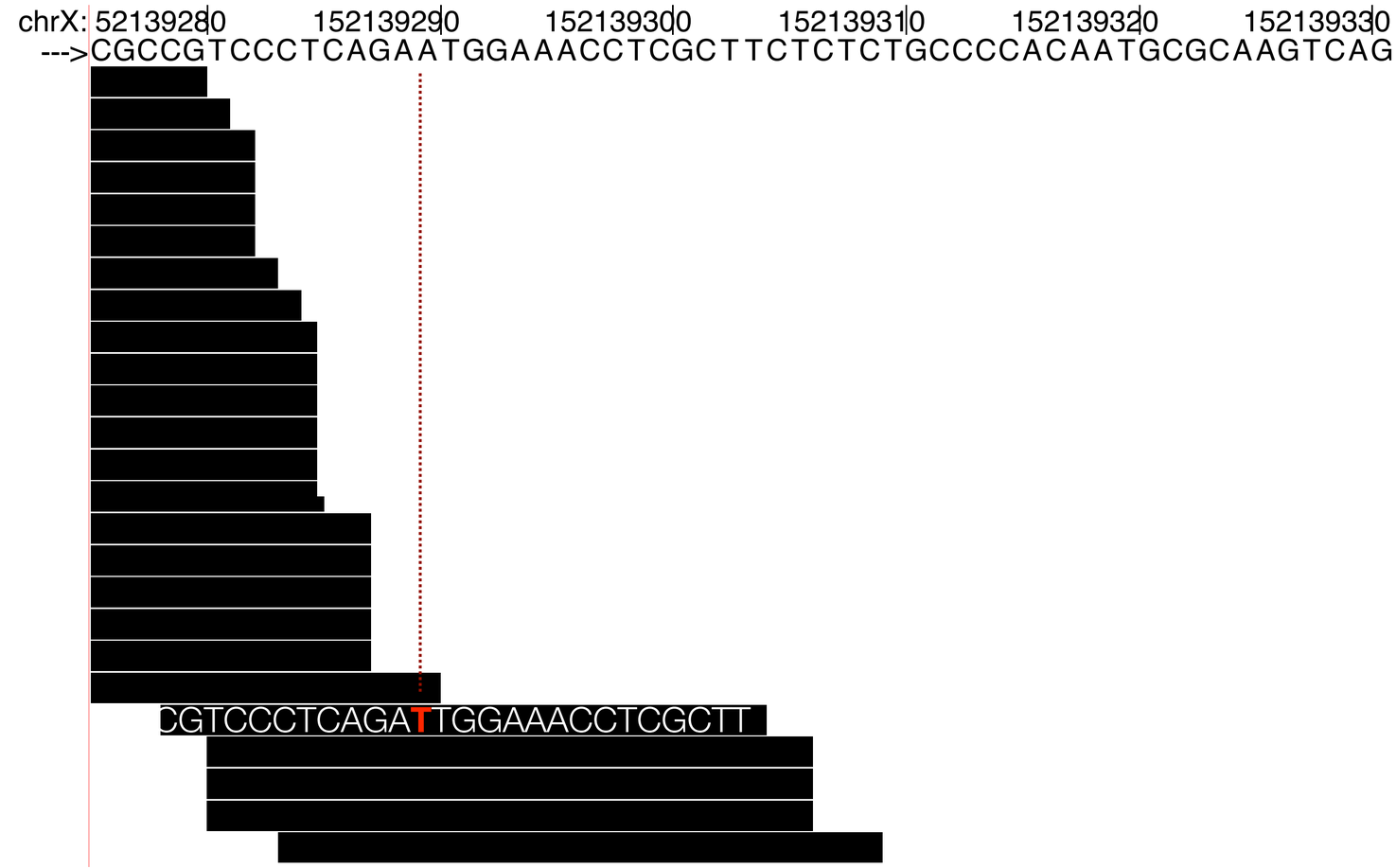
Genome

Sequence read

A simple case of string matching

Genome

Sequence reads



Difficult in practice

- Volume of data: ~3 Gbp
- ~50% of genome is **repeat regions** that cannot be covered by reads
  - Simple repeats, tandem, interspersed
  - Transposons
  - Segmental duplications where mapping is unclear
- **Gaps or unfinished regions**
  - peri-centromere, sub-telomere
  - ~5Mb unique to ethnic groups (e.g., African, Asian)

Challenges:
Human genome is large and complex

- Short reads: 50-150 bp (versus a very long reference)
  - Non-unique alignment
  - Sensitive to sequencing errors
- Massive amount of short reads: one lane produces ≥ 150 million 100 nucleotide reads
- Small insert size: 200-500 bp libraries

Challenges: short read NGS data

Reference	`ATCTCCATAGGACTAGAAGTAG`
Substitution	`ATCTCCATAGCACTAGAAGTAG`
Deletion	`ATCTCCATAGGAC-AGAAGTAG`
Insertion	`ATCTCCATAGGACTAGAAGTTAG`
3bp deletion	`ATCTC--AGGACTAGAAGTAG`

Challenges: non-exact matching

Local alignment vs Global alignment

- **Local alignment** matches the query with a *substring* (k-mer) of the reference
  - Tailored towards finding *regions of highly similar sequence* and aligning around those by working outwards to align the rest

Local Alignment

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

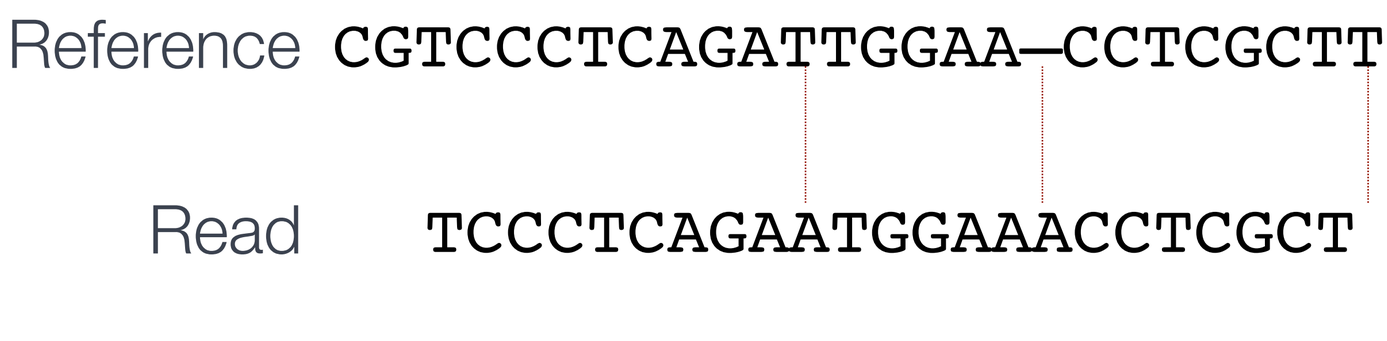
Global Alignment

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

- A **global alignment** performs end-to-end alignment between the query and the reference

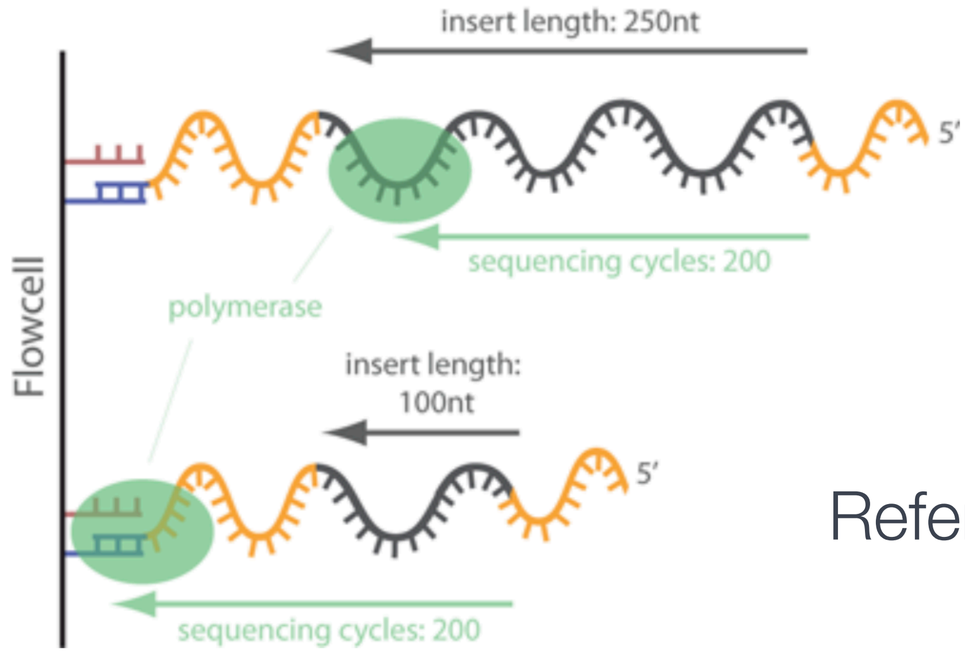
Reference	`ATCTCCATAGGACTAGAAGTAG`
Substitution	`ATCTCCATAGCACTAGAAGTAG`
Deletion	`ATCTCCATAGGAC-AGAAGTAG`
Insertion	`ATCTCCATAGGACTAGAAGTTAG`
3bp deletion	`ATCTC--AGGACTAGAAGTAG`

General concepts: edit distance



Edit distance =3

General concepts: edit distance



Reference `CGTCCCTCAGATTGGAAACCTCGCTT`

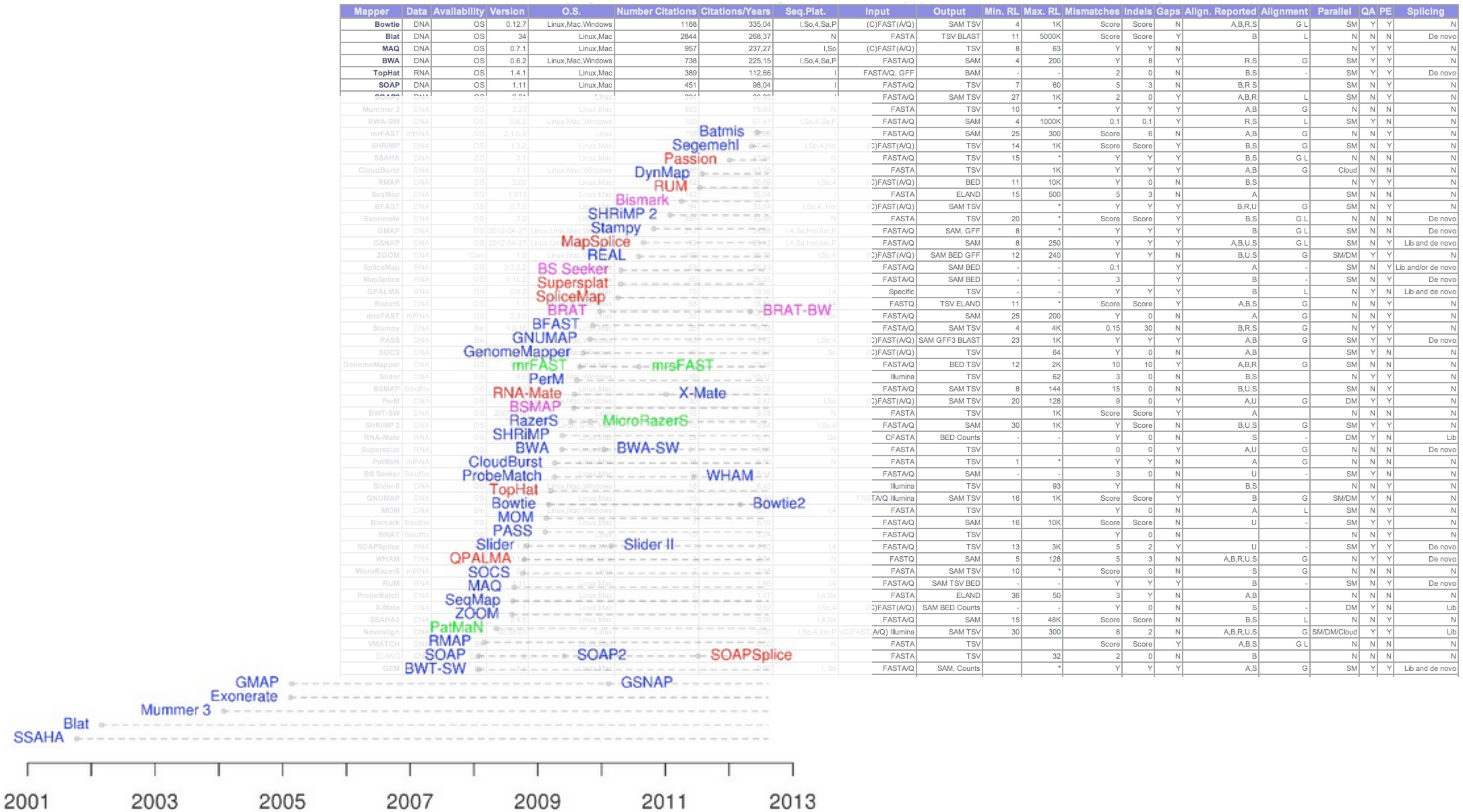
Read `AGGCTACGATTGGAAACCTCGCTT`

General concepts: soft-clipping

Image source: ECSEQ Bioinformatics

Soft-clipping of reads

- Portions of the read that do not match well to the reference genome on either side of the reads are ignored for the alignment
- The procedure can carry a small penalty for each soft-clipped base, but amounts to a significantly smaller penalty than mismatching bases
- Soft-clipped bases are retained in the sequence and simply marked. Trimming methods hard-clip, which deletes the unwanted sequence.



Short-read aligners: choices

http://wwwdev.ebi.ac.uk/fg/hts_mappers/

Building an index

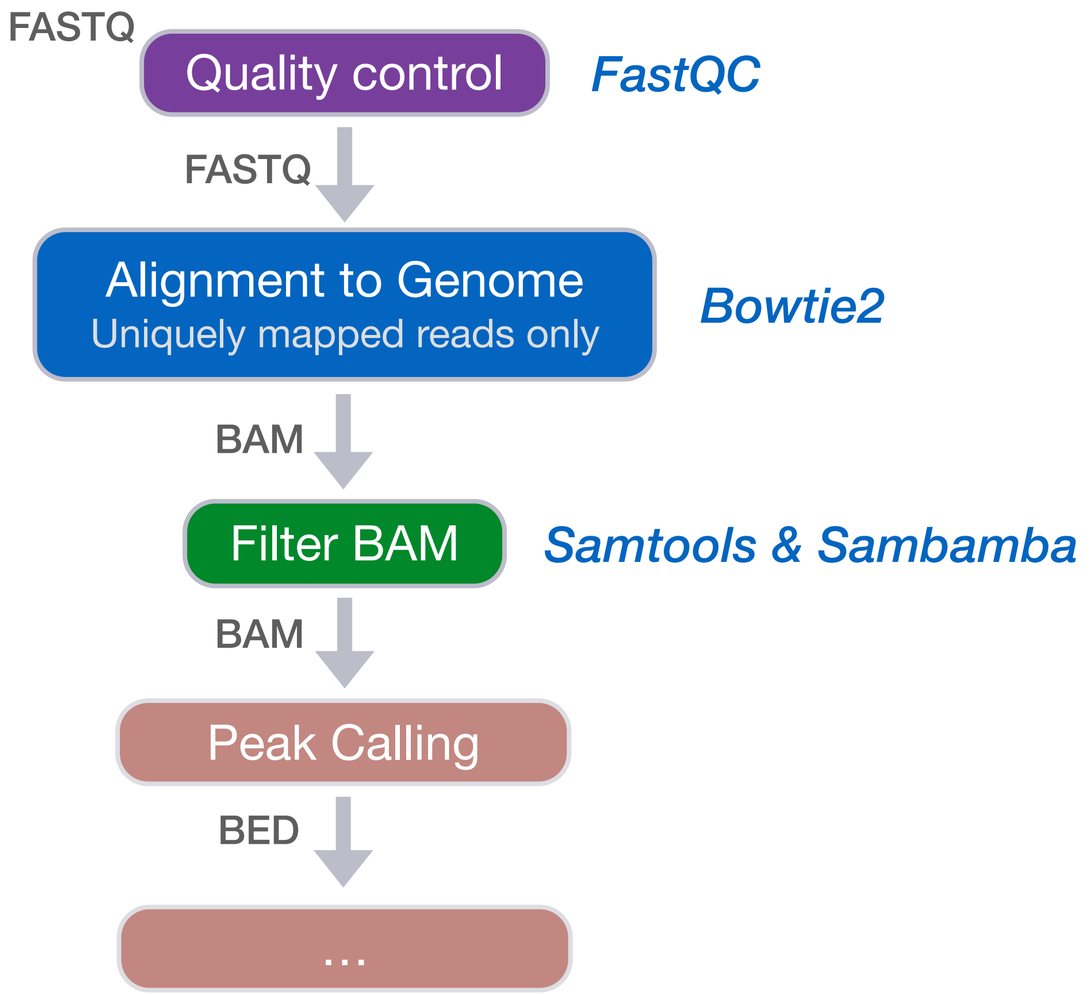
- Having an index of the reference genome provides an efficient way to search
- Once index is built, it can be queried any number of times
- Indexes are genome and tool-specific
- Different types of indices (i.e hash-tables, suffix arrays, Burrows-Wheeler Transform)

- Bowtie2: indexes with an FM Index to keep low memory footprint. Supports gapped, local and paired-end alignment
- BWA: indexes with the Burrows-Wheeler Transform (BWT). Has three algorithms for varying read lengths.
- SOAP2: uses a 2-way BWT for indexing. Fast and accurate alignment of Illumina sequencing reads. Not open source.
- MAQ: first aligns reads to reference sequences and then calls the consensus. Designed for Illumina reads.

Commonly used aligners for ChIP-seq

Alignment considerations for ChIP-seq

- Percentage of mapped reads
    - 75% or higher is good; Percentages vary between organisms
- Duplicates
    - Usually due to over-amplification or short read length
    - ENCODE guidelines suggest < 20% duplication rate with paired-end data; can be more lenient with single-end
- Multi-mappers
    - Reduce by setting allowable mismatches according to sequencing platform
    - For situations when protein binds repetitive DNA, use paired-end sequencing



ChIP-seq: Alignment and Filtering

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