

Aligning reads: tools and theory

Genome

chrX: 52139280 152139290 152139300 152139310 152139320 152139330
--->CGCCGTCCCTCAGAAATGGAAACCTCGCTTCTCTCTGCCCCACAATGCGCAAGTCAG

Sequence read

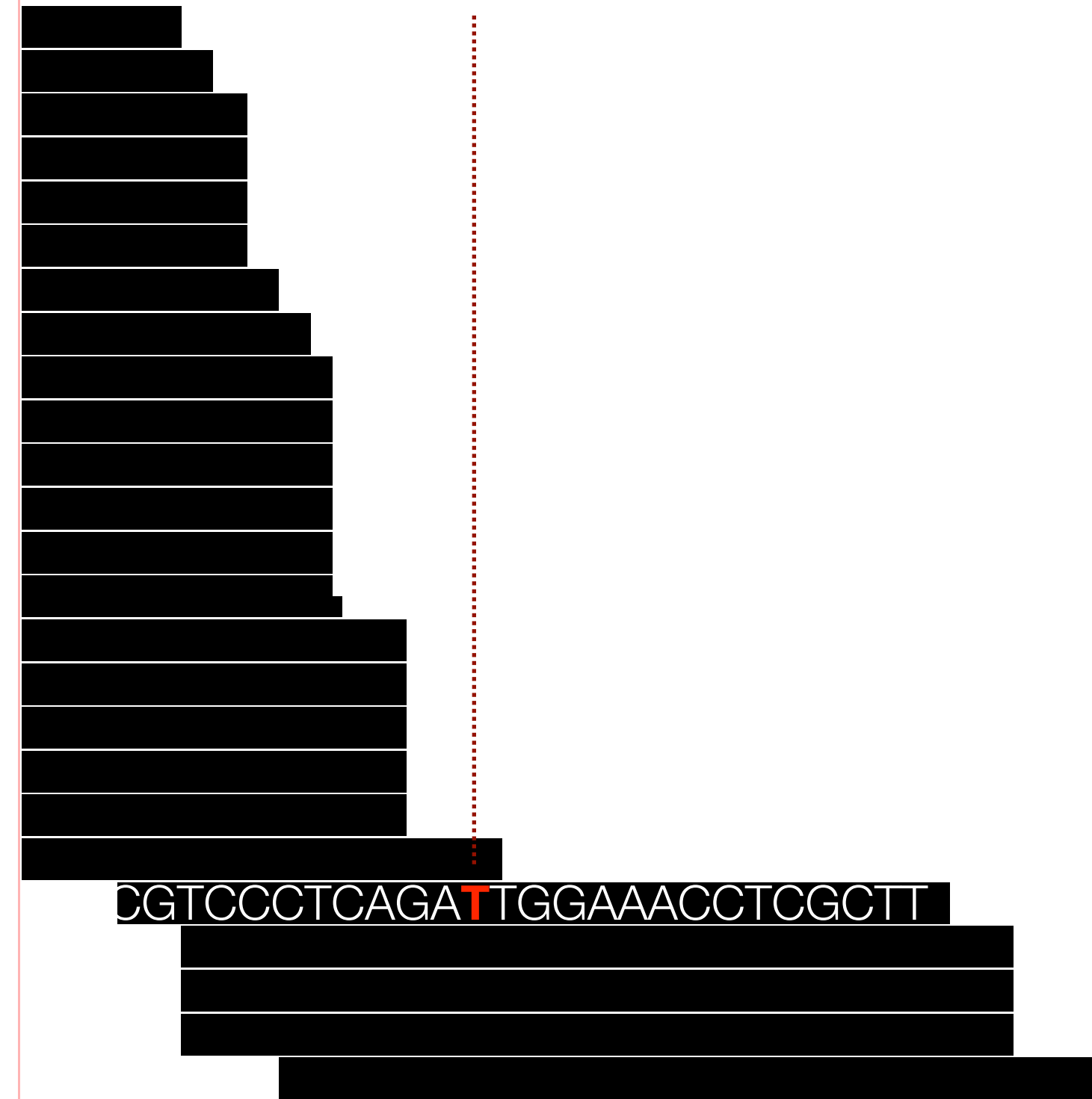
CGTCCCTCAGAAATGGAAACCTCGCTT

A simple case of string matching

Genome

chrX: 52139280 152139290 152139300 152139310 152139320 152139330
--->CGCCGTCCCTCAGAAATGGAAACCTCGCTTCTCTCTGCCCCACAATGCGCAAGTCAG

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 ATCTCCATAG**C**ACTAGAAGTAG

Deletion ATCTCCATAGGAC**-**AGAAGTAG

Insertion ATCTCCATAGGACTAGAAGT**T**AG

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 ATCTCCATAGGACTAGGAAGTAG

Substitution ATCTCCATAG**C**ACTAGGAAGTAG

Deletion ATCTCCATAGGAC**-**AGGAAGTAG

Insertion ATCTCCATAGGACTAGGAAGT**T**AG

3bp deletion ATCTC**---**AGGACTAGGAAGTAG

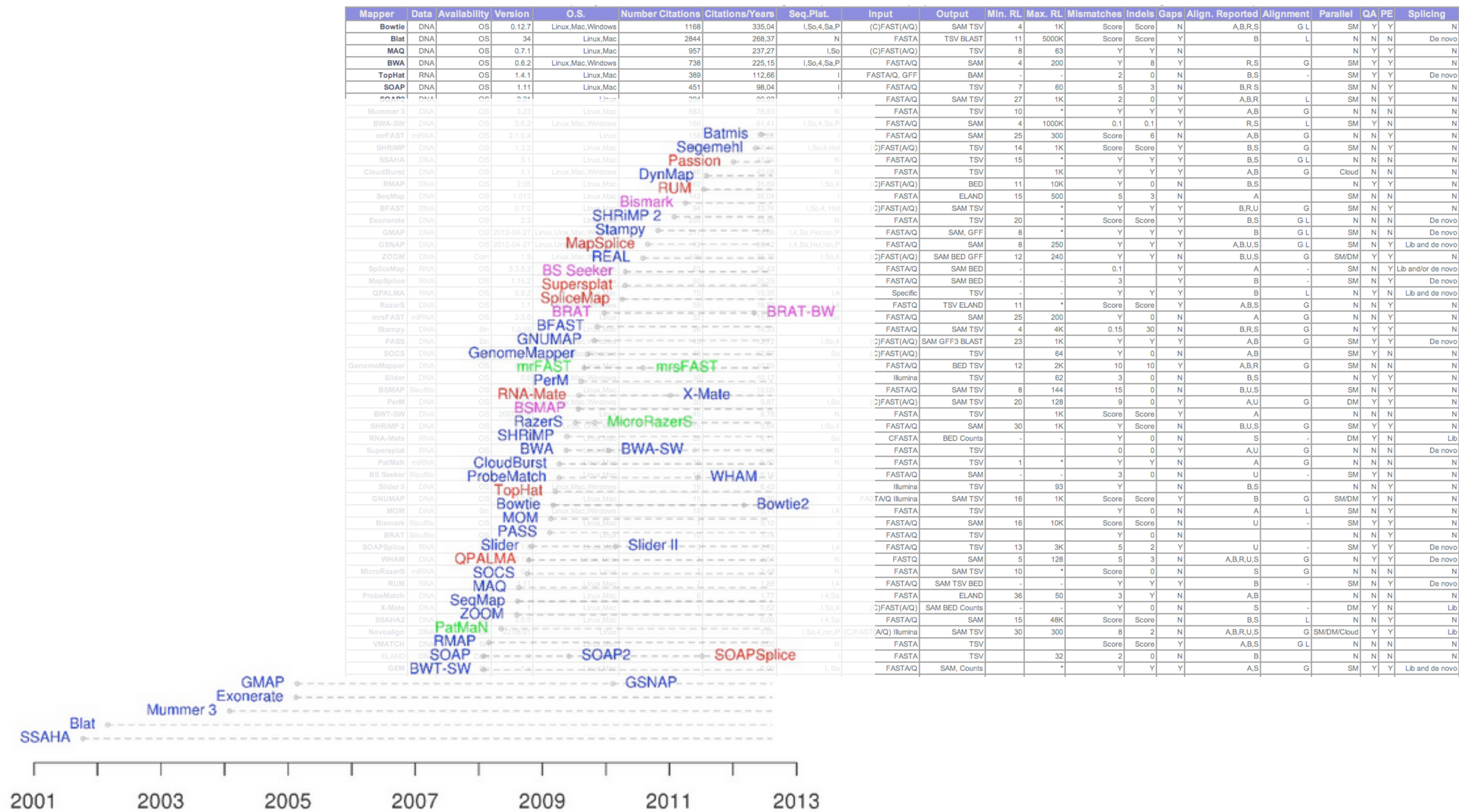
General concepts: edit distance

Reference CGTCCCTCAGATTGGAA—CCTCGCTT

Read TCCCTCAGAATGGAAACCTCGCT

Edit distance =3

General concepts: edit distance



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)



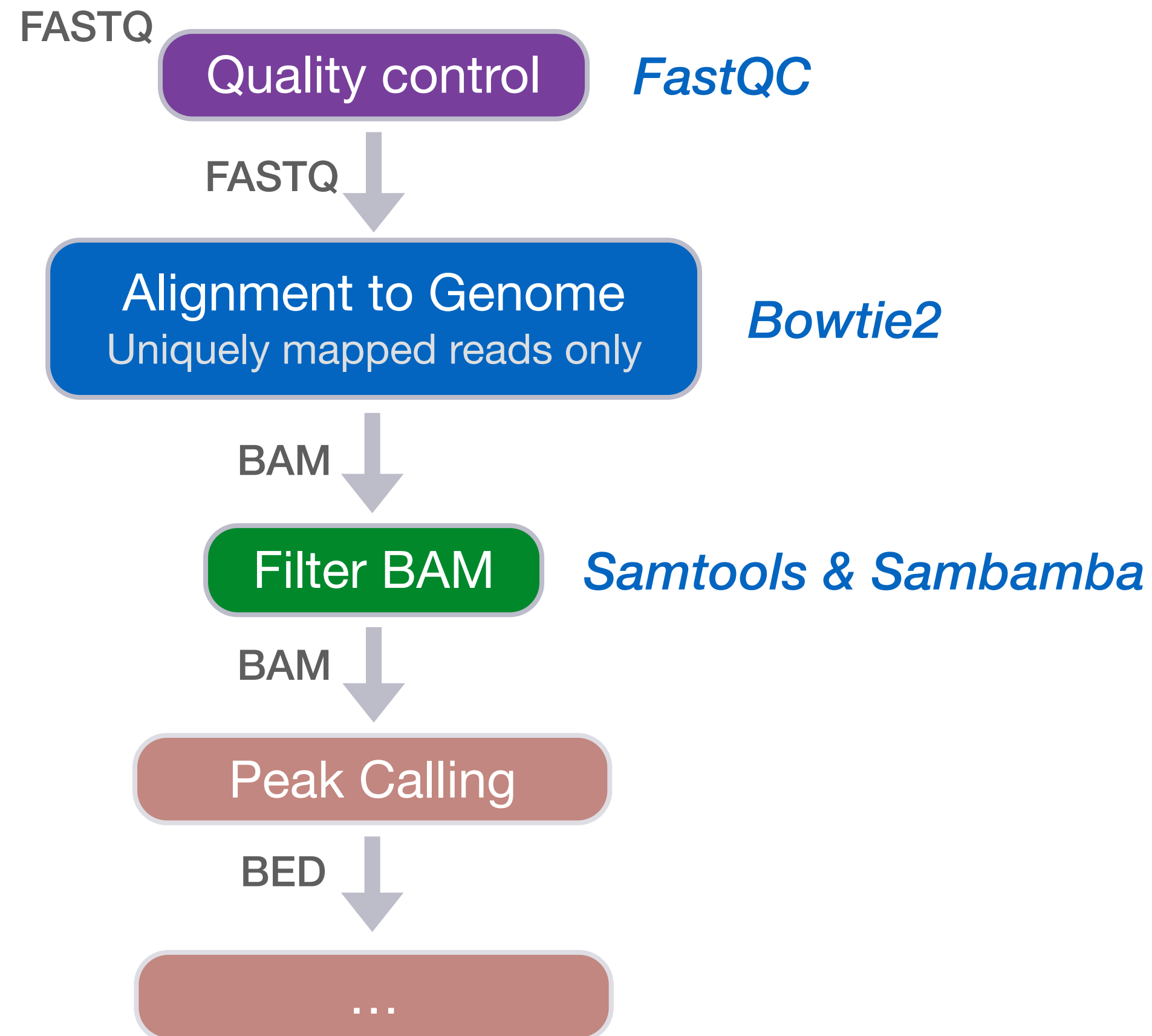
- ▶ Bowtie2: indexes with an FM Index to keep low memory footprint. Supports gapped, local and paired-end alignment
- ▶ BWA: indexes with the Burrows-Wheeler Transform (BWT). Has three algorithms for varying read lengths.
- ▶ SOAP2: uses a 2-way BWT for indexing. Fast and accurate alignment of Illumina 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 uniquely mapped reads
 - ▶ 70% or higher is good. 50% or lower is concerning.
 - ▶ Percentages vary between organisms
- ▶ Duplicates
 - ▶ Usually due to over-amplification or short read length
- ▶ Multi-mappers
 - ▶ Reduce by setting allowable mismatches according to sequencing platform
 - ▶ For situations when protein binds repetitive DNA, use paired-end sequencing

Keep only uniquely mapped reads for peak calling!



ChIP-seq: Alignment and Filtering

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