Aligning new-sequencing reads by BWA

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Outline

1. Short-read alignment
   - Overview of read alignment
   - Short-read aligners
2. BWA: Burrows-Wheeler Aligner
   - Overview of BWA
   - Running BWA

Outline

Overview of read alignment

- Alignment and *de novo* assembly are the first steps once read sequences are obtained.
- The task: to align sequencing reads against a known reference sequence for variation discovery (SNPs, indels and CNVs), ChIP-seq or RNA-seq.
- Difficulties: efficiency and ambiguity caused by repeats and sequencing errors.
- Aligners for long reads (>200bp): BLAT, SSAHA2 and BWA-SW.

There are many short-read aligners...

- Bfast
- BioScope
- Bowtie
- BWA
- CLC bio
- CloudBurst
- Eland/Eland2
- GenomeMapper
- GnuMap
- Karma
- MAQ
- MOM
- Mosaik
- MrFAST/MrsFAST
- NovoAlign
- PASS
- PerM
- RazerS
- RMAP
- SSAHA2
- Segemehl
- SeqMap
- SHRiMP
- Slider/SliderII
- SOAP/SOAP2
- Srprism
- Stampy
- vmatch
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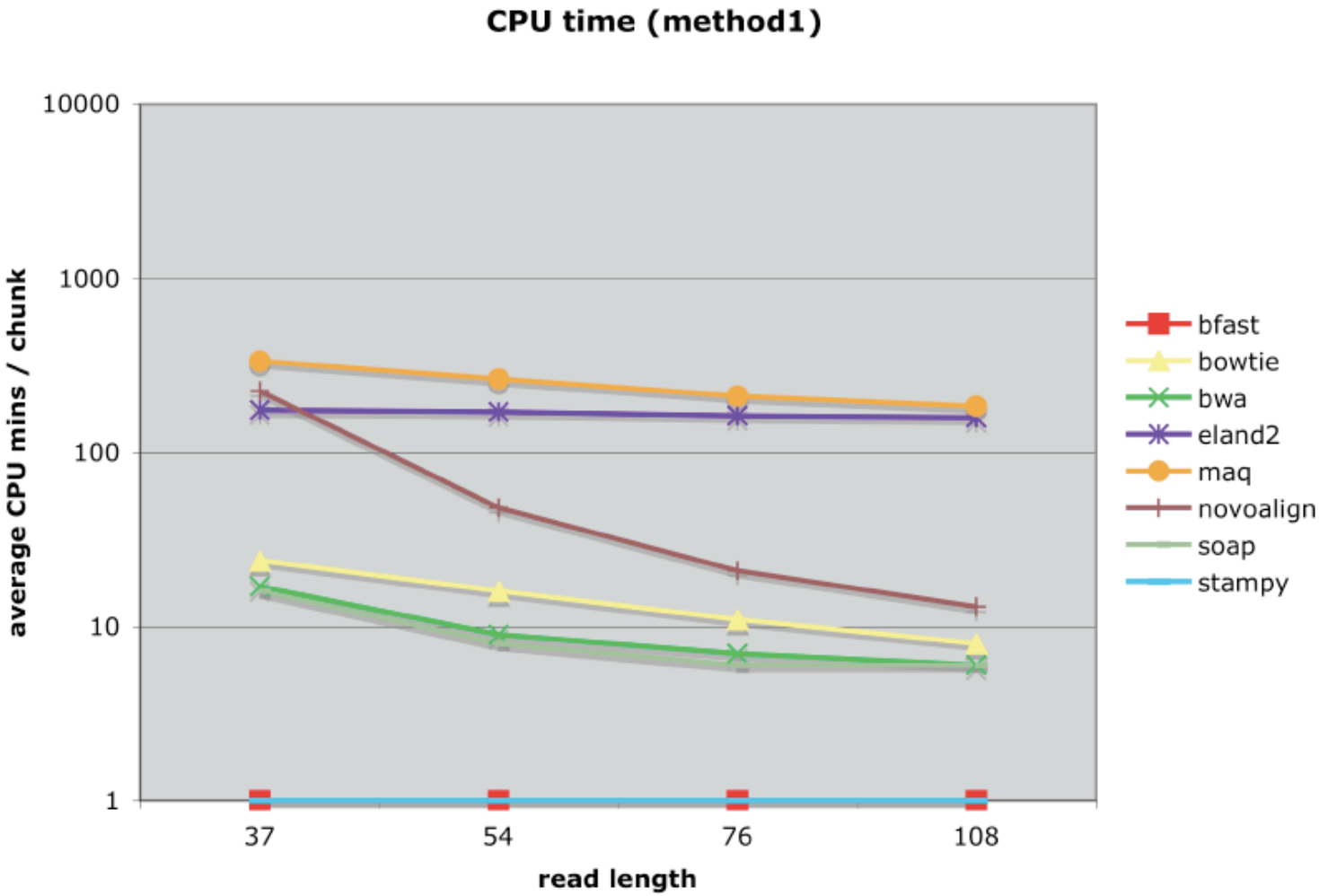
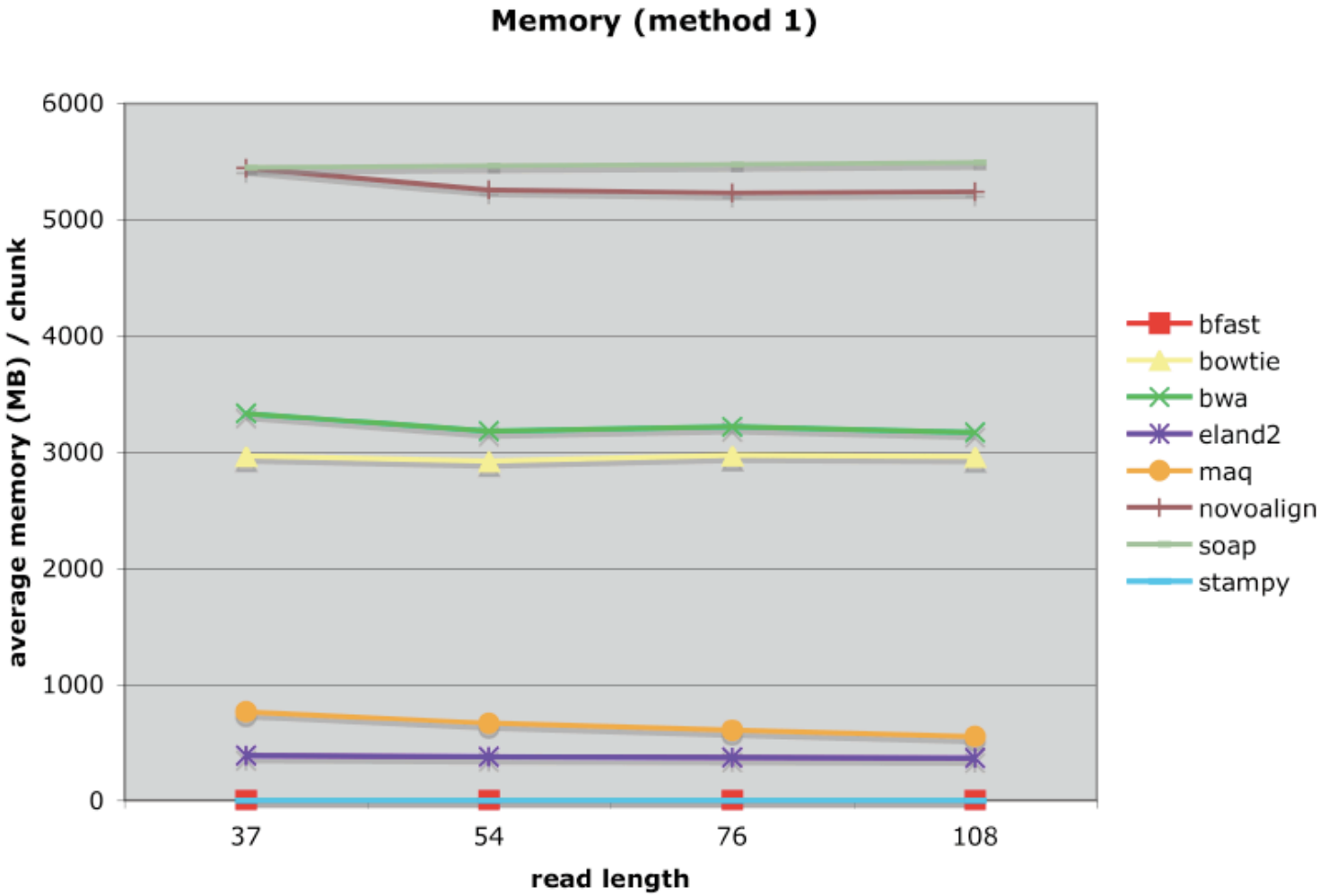
The speed varies...



by Bala *et al.*

The memory varies...



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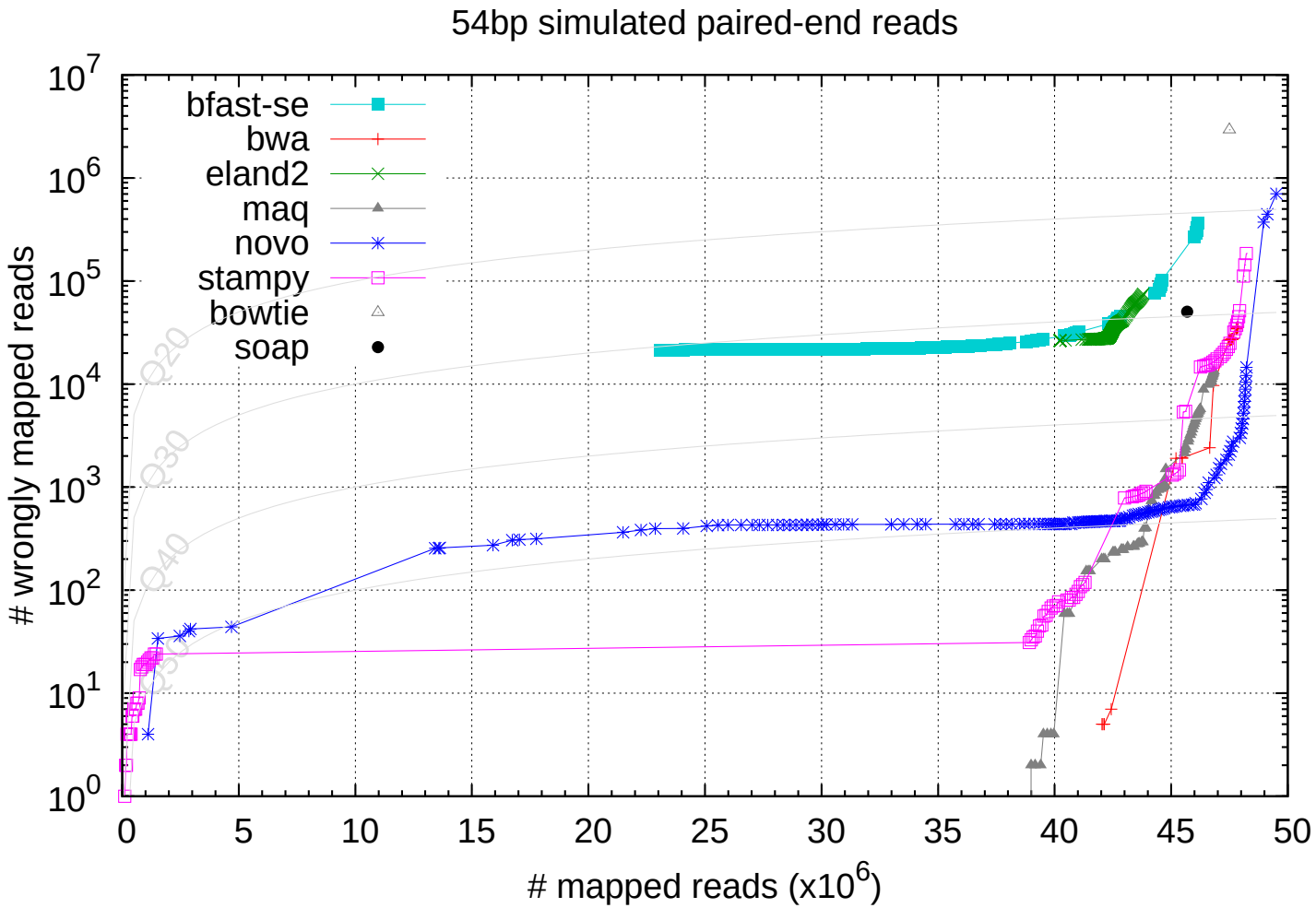
The accuracy varies...



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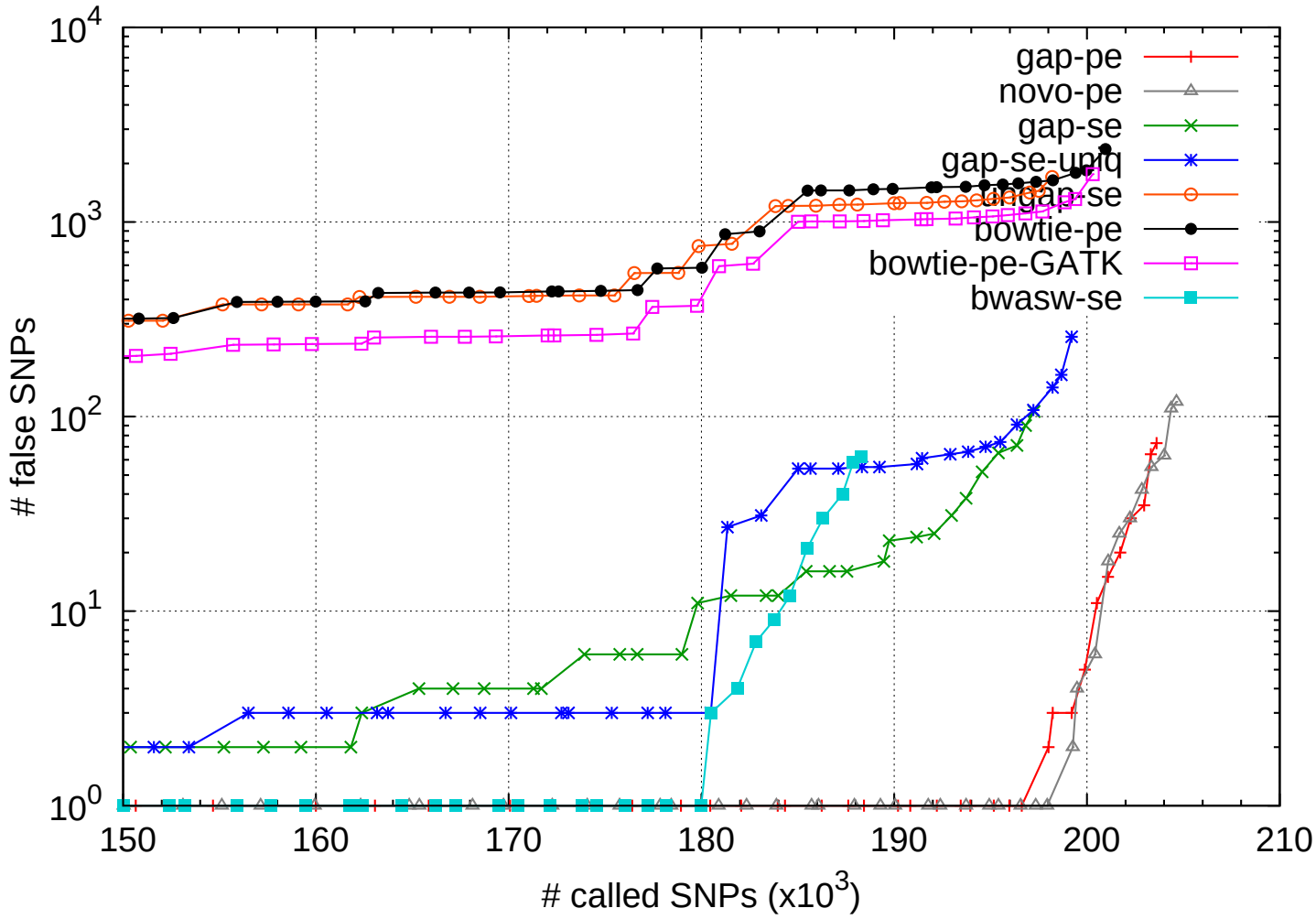
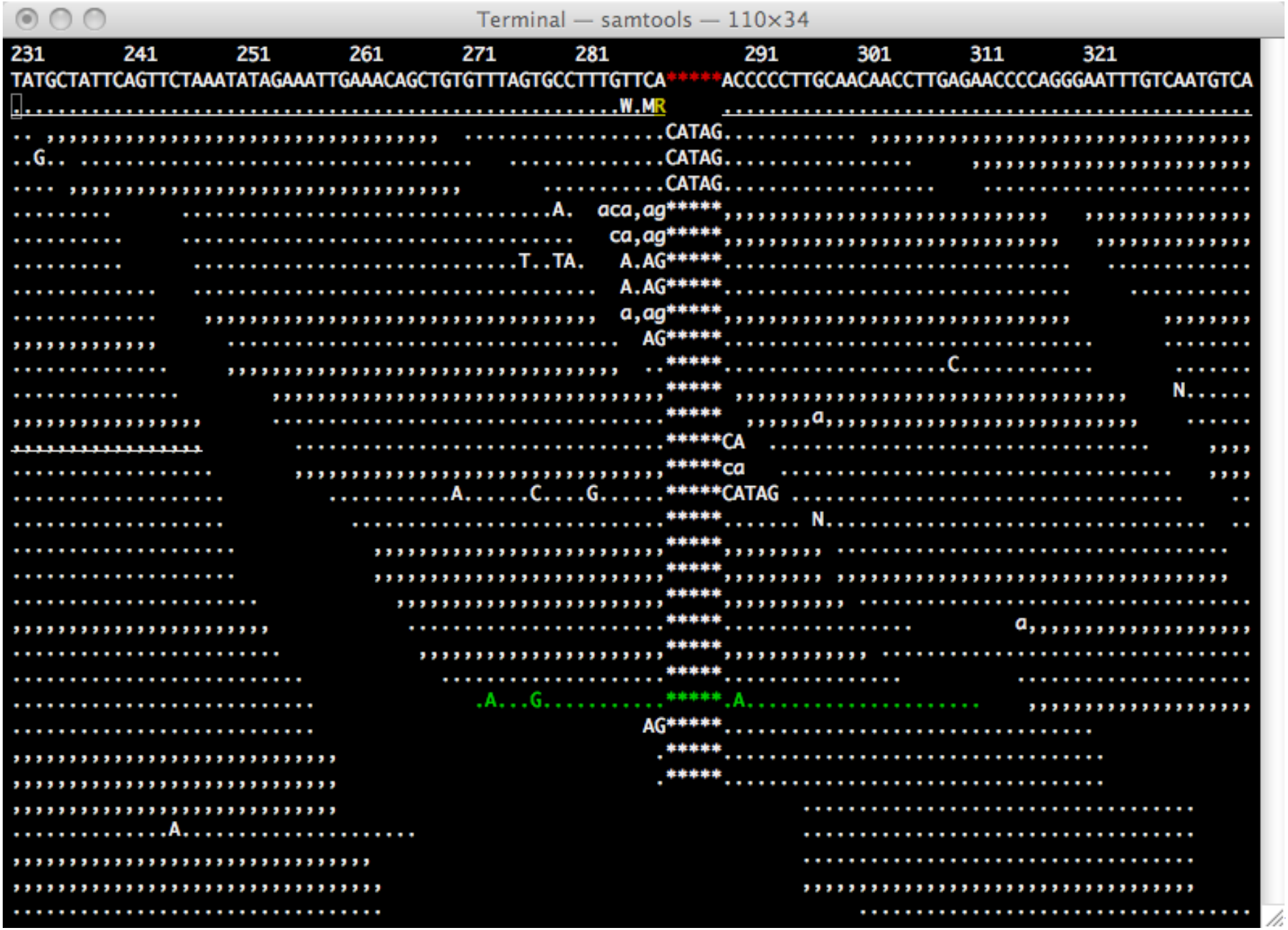
Alignment strategy and SNP calling




Terminal — samtools — 110×34
231 241 251 261 271 281 291 301 311 321
TATGCTATTCAGTTCTAAATATAGAAATTGAAACAGCTGTGTTTAGTGCCTTTGTTCA*****ACCCCCTTGCAACAACCTTGAGAACCCCAGGGAATTTGTCAATGTCA

Choosing aligners

- There are many aligners and they vary a lot in performance.
- Aligners also vary in accuracy.
- Alignment accuracy is likely to affect the identification of structural variations (SVs), depending on algorithms though.
- In SNP calling, effective pair-end mapping and gapped alignment are essential to high SNP accuracy.

BWA is carefully designed to achieve a good balance between performance and accuracy.

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Outline

Overview of the BWA algorithm

- Based on FM-index (Burrows-Wheeler Transform plus auxillary data structures) which enables fast exact matching.
- Short-read algorithm: alter the read sequence such that it matches the reference exactly.
- Long-read algorithm (BWA-SW): sample reference subsequences and perform Smith-Waterman alignment between the subsequences and the read.
- Work for Illumina and SOLiD single-end (SE) and paired-end (PE) reads; new component BWA-SW for 454/Sanger SE reads.

Key features

- Fast and moderate memory footprint (<4GB)
- SAM output by default
- Gapped alignment for both SE and PE reads
- Effective pairing to achieve high alignment accuracy; suboptimal hits considered in pairing.
- Non-unique read is placed randomly with a *mapping quality* 0; all hits can be outputted in a concise format.
- Guarantee to find k-difference in the seed (first 32bp by default).
- The default configuration works for most typical input.
  - Automatically adjust parameters based on read lengths and error rates.
  - Estimate the insert size distribution on the fly.

Running BWA

- Input: ref.fa, read1.fq.gz, read2.fq.gz and long-read.fq.gz
- Step 1: Index the genome (∼3 CPU hours for the human genome):

```
bwa index -a bwtsw ref.fa
```

- Step 2a: Generate alignments in the suffix array coordinate:

```
bwa aln ref.fa read1.fq.gz > read1.sai
bwa aln ref.fa read2.fq.gz > read2.sai
```

  Apply option `-q15` if the quality is poor at the 3'-end of reads.

- Step 3a: Generate alignments in the SAM format:

```
bwa sampe ref.fa read?.sai read?.fq.gz > aln.sam
```

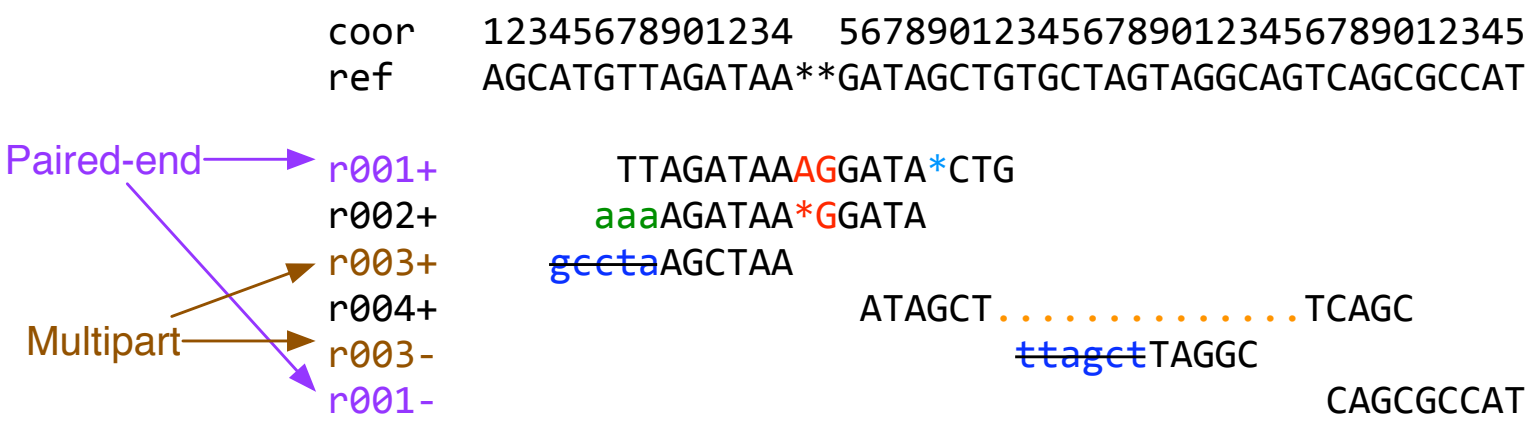
- Step 4a: Get multiple hits:

```
bwa samse -n 100 ref.fa read1.sai read1.fq.gz
```

- Step 2b: Use BWA-SW for long reads:

```
bwa bwasw ref.fa long-read.fq.gz > aln-long.sam
```

The Sequence Alignment/Map format



Ins & padding
Soft clipping
Splicing
Hard clipping

```
@SQ SN:ref LN:45
r001 163 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTA *
r002   0 ref  9 30 3S6M1P1I4M * 0   0 AAAAGATAAGGATA     *
r003   0 ref  9 30 5H6M       * 0   0 AGCTAA    *   NM:i:1
r004   0 ref 16 30 6M14N5M    * 0   0 ATAGCTTCAGC       *
r003  16 ref 29 30 6H5M       * 0   0 TAGGC     *   NM:i:0
r001  83 ref 37 30 9M         = 7 -39 CAGCGCCAT         *
```

```
ref  7 T 1 .    | ref 12 T 3 ...        | ref 17 T 3 ...
ref  8 T 1 .    | ref 13 A 3 ...        | ref 18 A 3 .-1G..
ref  9 A 3 ...  | ref 14 A 2 .+2AG.+1G. | ref 19 G 2 *.
ref 10 G 3 ...  | ref 15 G 2 ..         | ref 20 C 2 ..
ref 11 A 3 ..C  | ref 16 A 3 ...        | ...
```

Acknowledgement

- Richard Durbin and the Durbin research group
- All BWA users