TOPIC 7:

Read Mapping

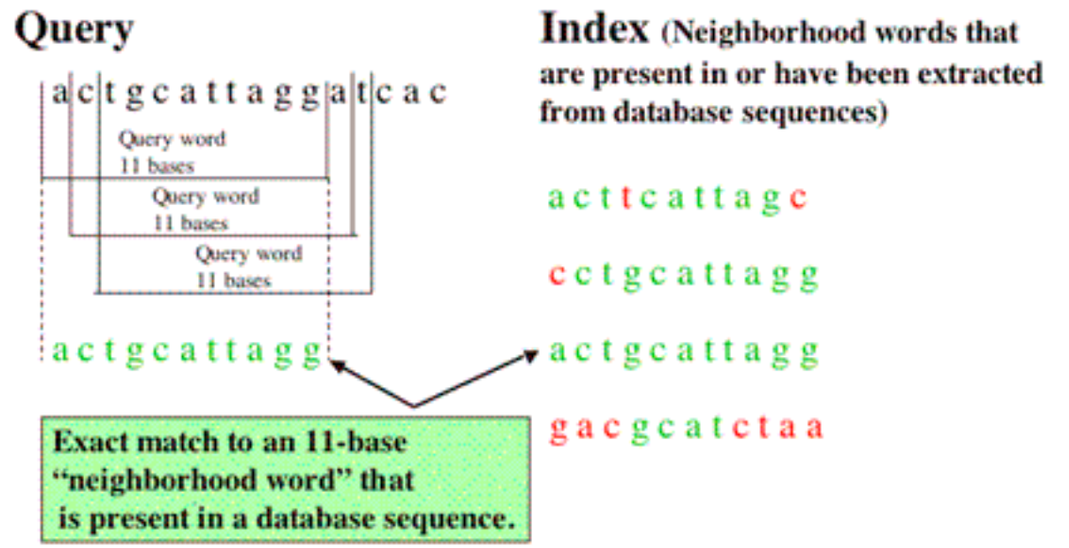
Outline

- Algorithm at the heart of the dominant short read mapper
  - Suffix-trie structures
- Long read alignment - challenges and approaches
- Anatomy of the BAM/SAM file format

BLAST - Best Local Alignment Search Tool

Why not use BLAST for short read data?

- Typically takes 0.1 to 1 second to search 1 sequence against a database
- 60 million reads equates to ~70 CPU days



Approaches to align reads

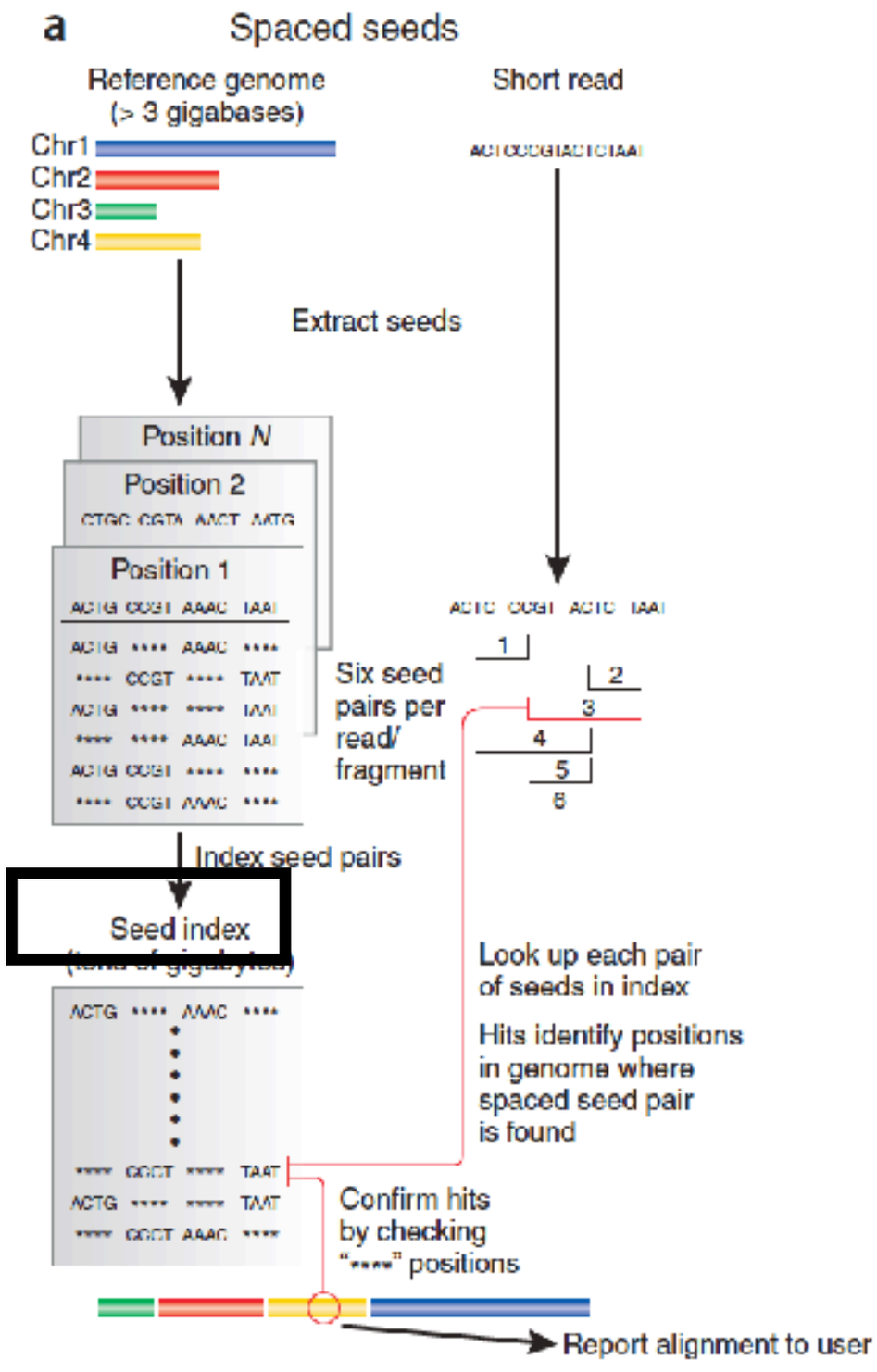


Figure modified from Trapnell & Salzberg 2009

Hashed seed-extend algorithms

Builds a hash of seeds from the reference genome

Then a two step process:

- Identify a match to the seed sequence in the reference

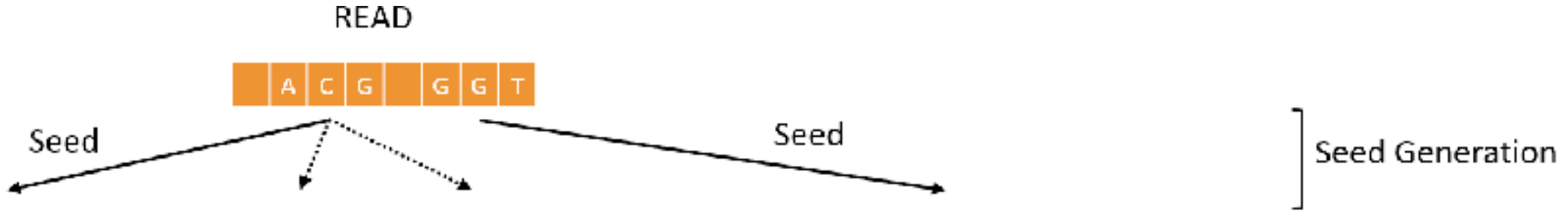
- Extend match using sensitive (but slow) Smith-Waterman algorithm

Seed-extend algorithm

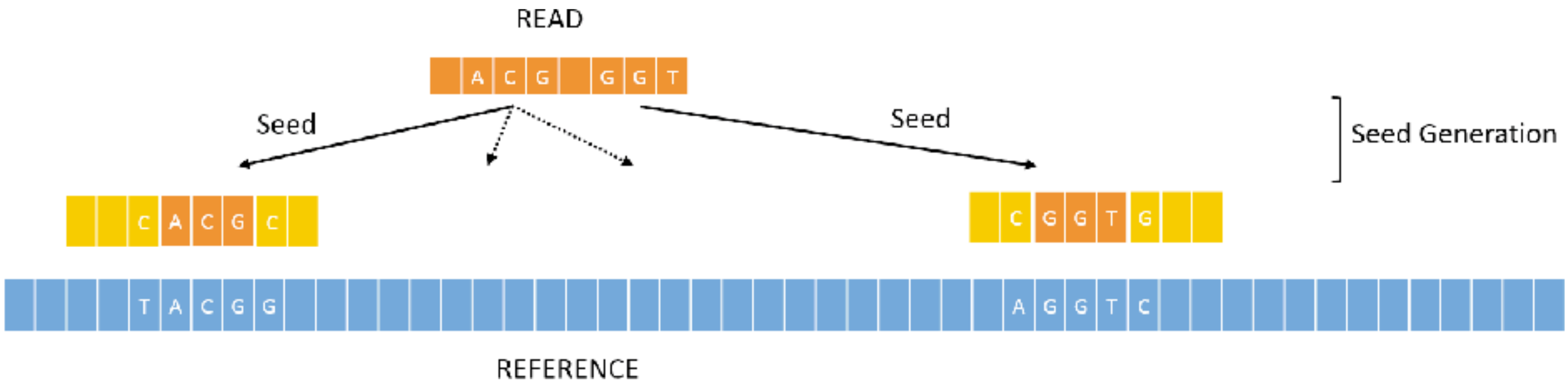
READ

REFERENCE

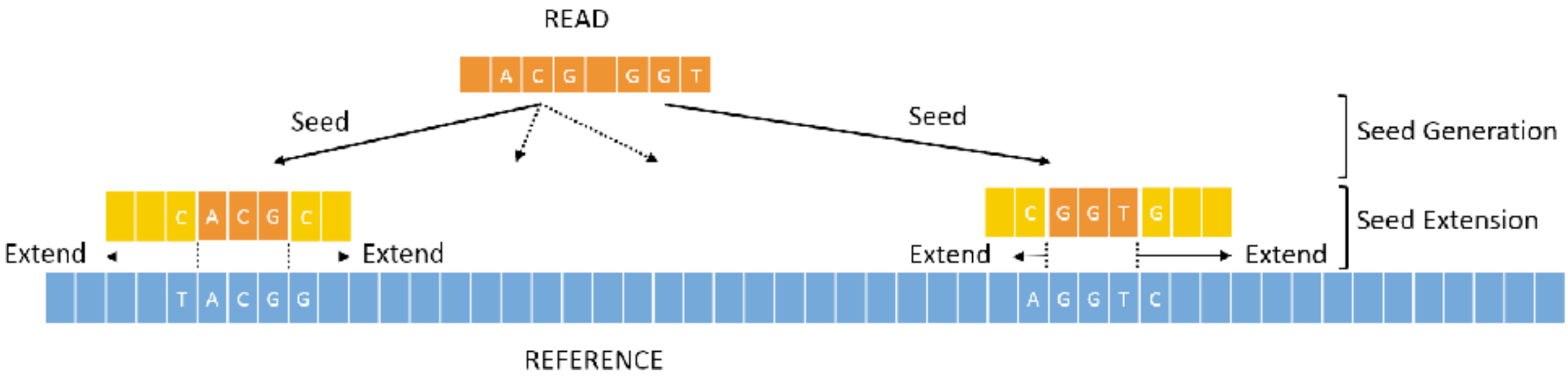
Seed-extend algorithm



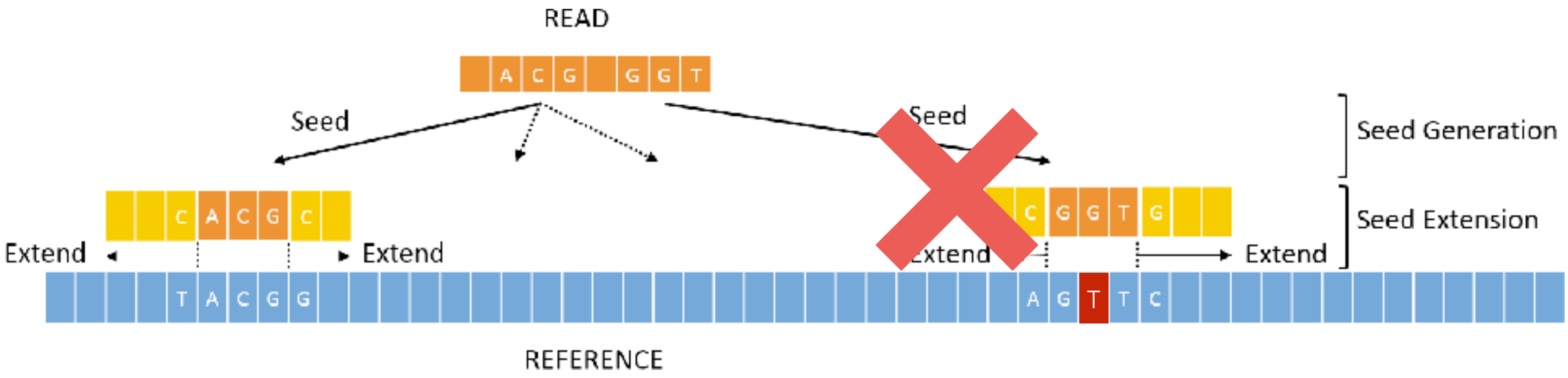
Seed-extend algorithm



Seed-extend algorithm



Seed-extend algorithm



Spaced seeds

To increase sensitivity we can used spaced-seeds:

```
111111111          Consecutive seed template with length 9bp
GATAGCTAGCTAAT     Reference
  AGCTAGCTA        Query
```

```
101011010111011    Consecutive seed template with weight 9bp
GATAGCTAGCTAAT     Reference
GATAGCGAGCTAAT     Query
```

Spaced seeds

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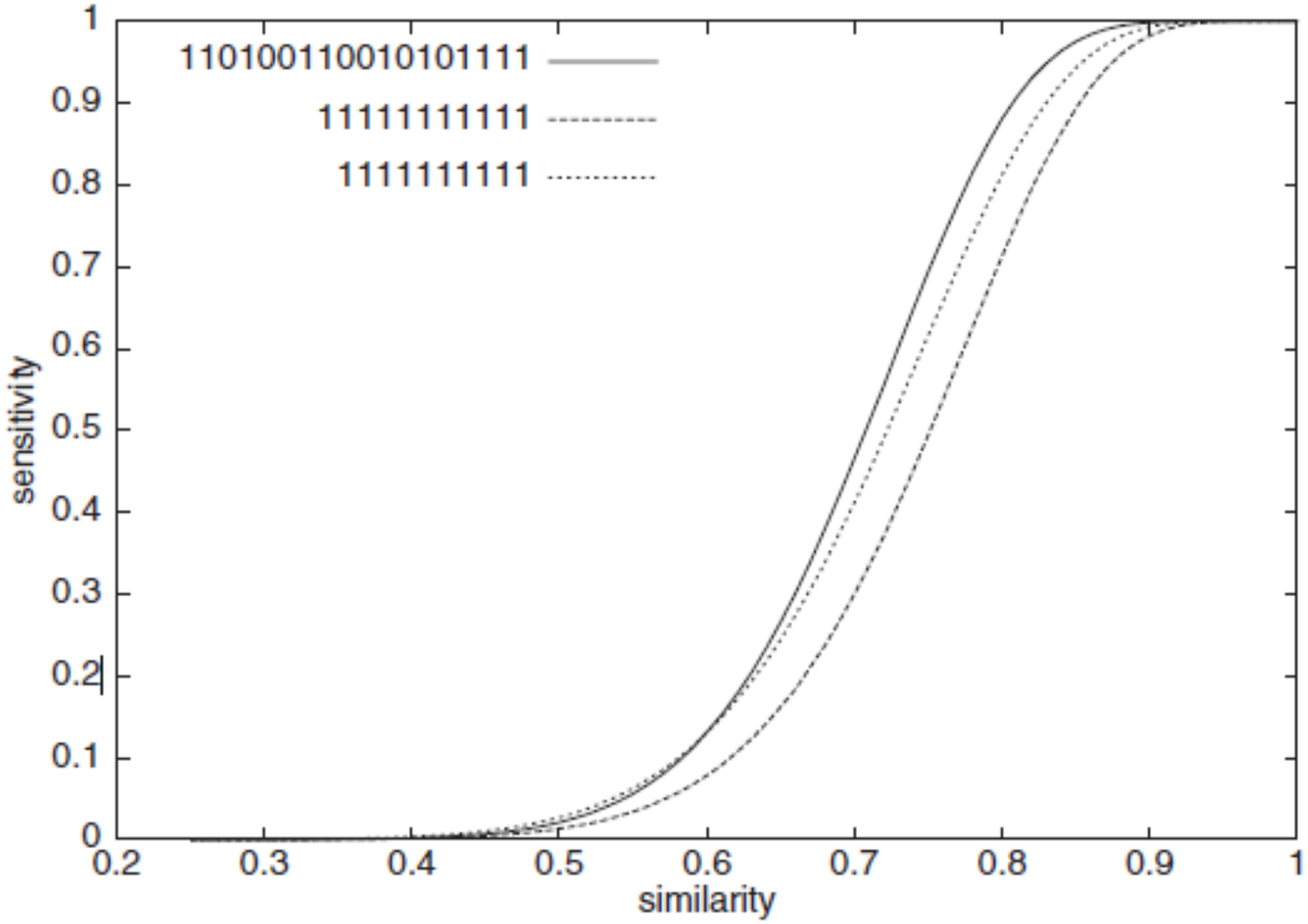


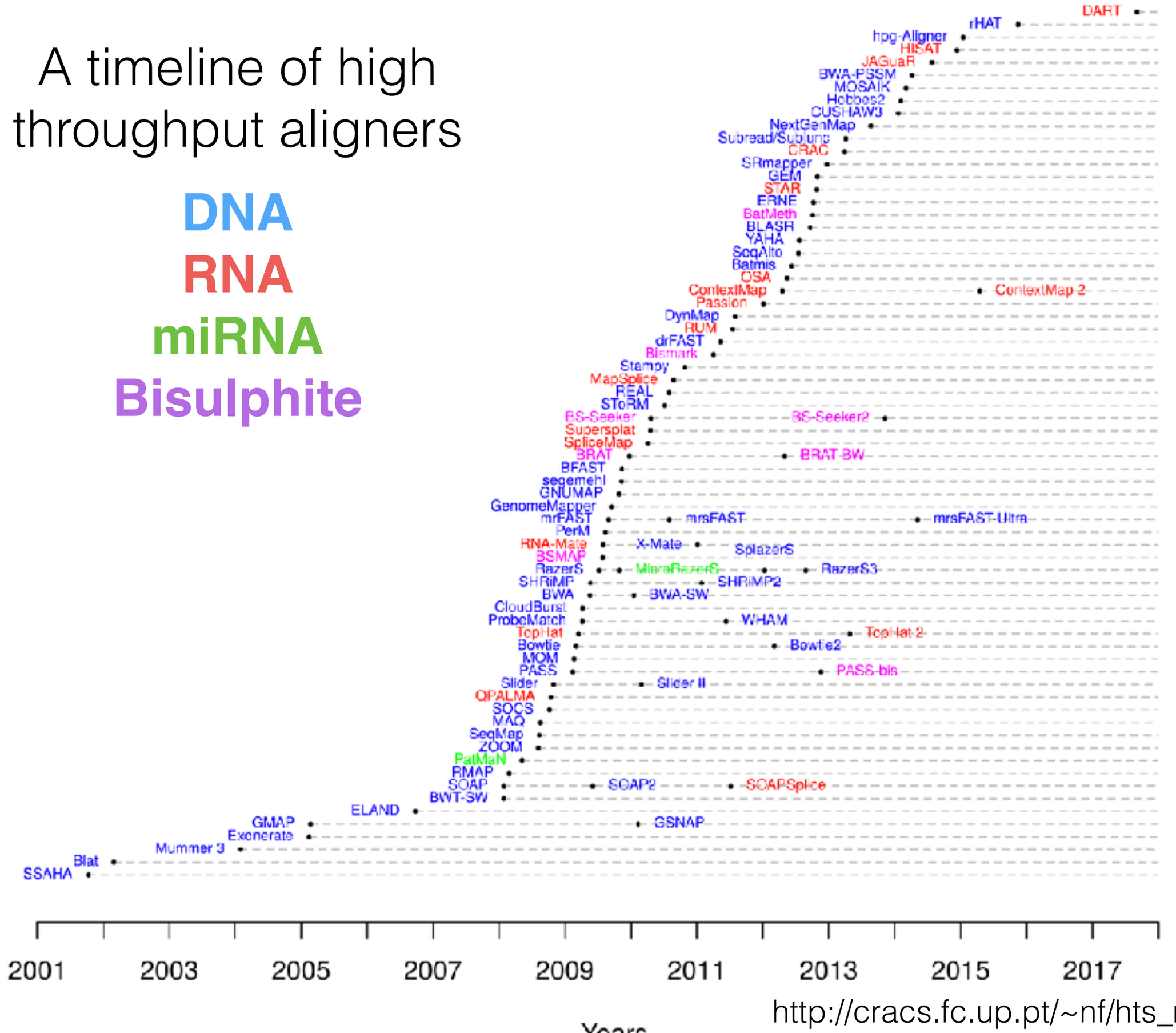
A timeline of high throughput aligners

DNA

RNA

miRNA

Bisulphite



http://cracs.fc.up.pt/~nf/hts_mappers/

Approaches to align short reads

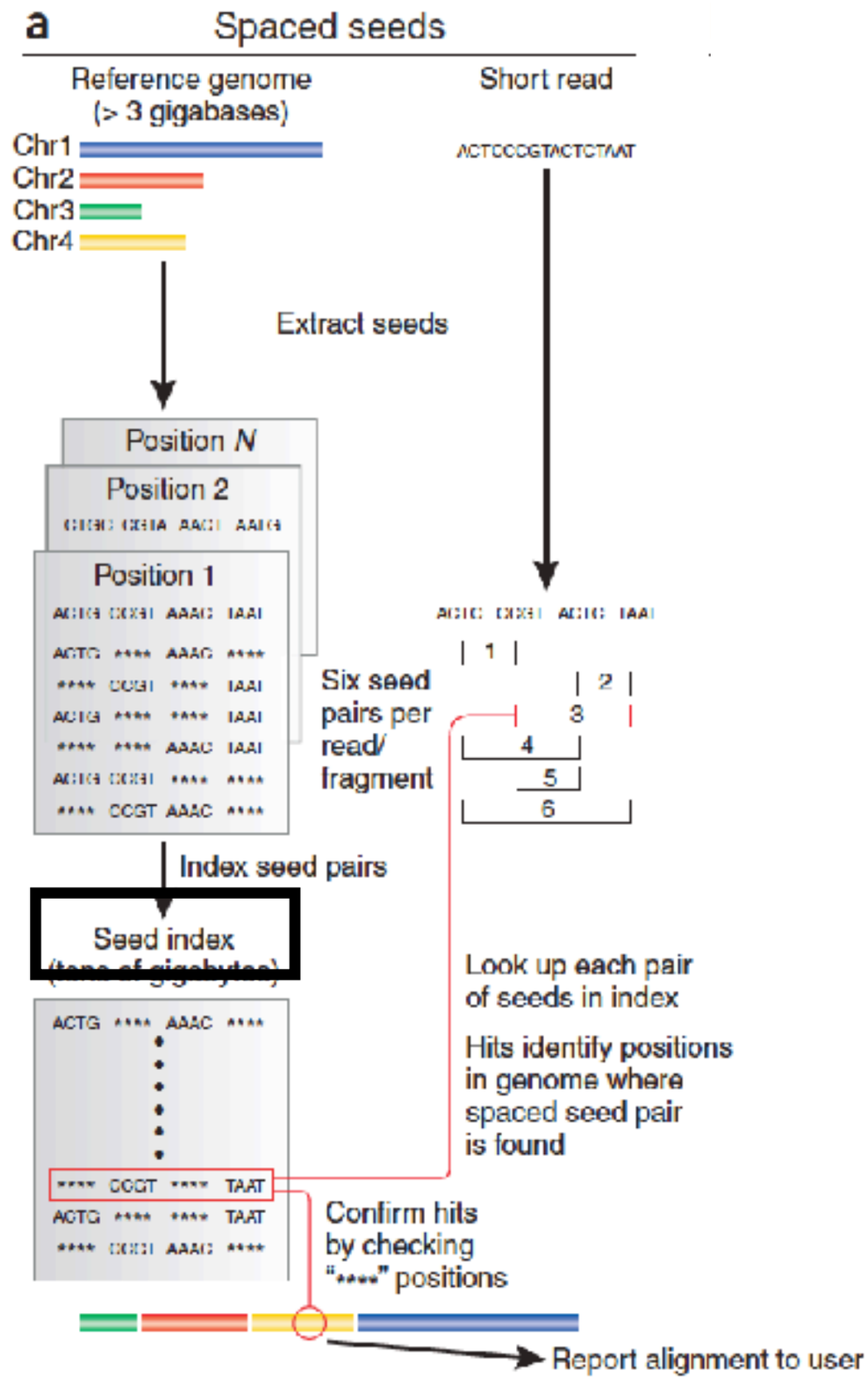


Figure modified from Trapnell & Salzberg 2009

Approaches to align short reads

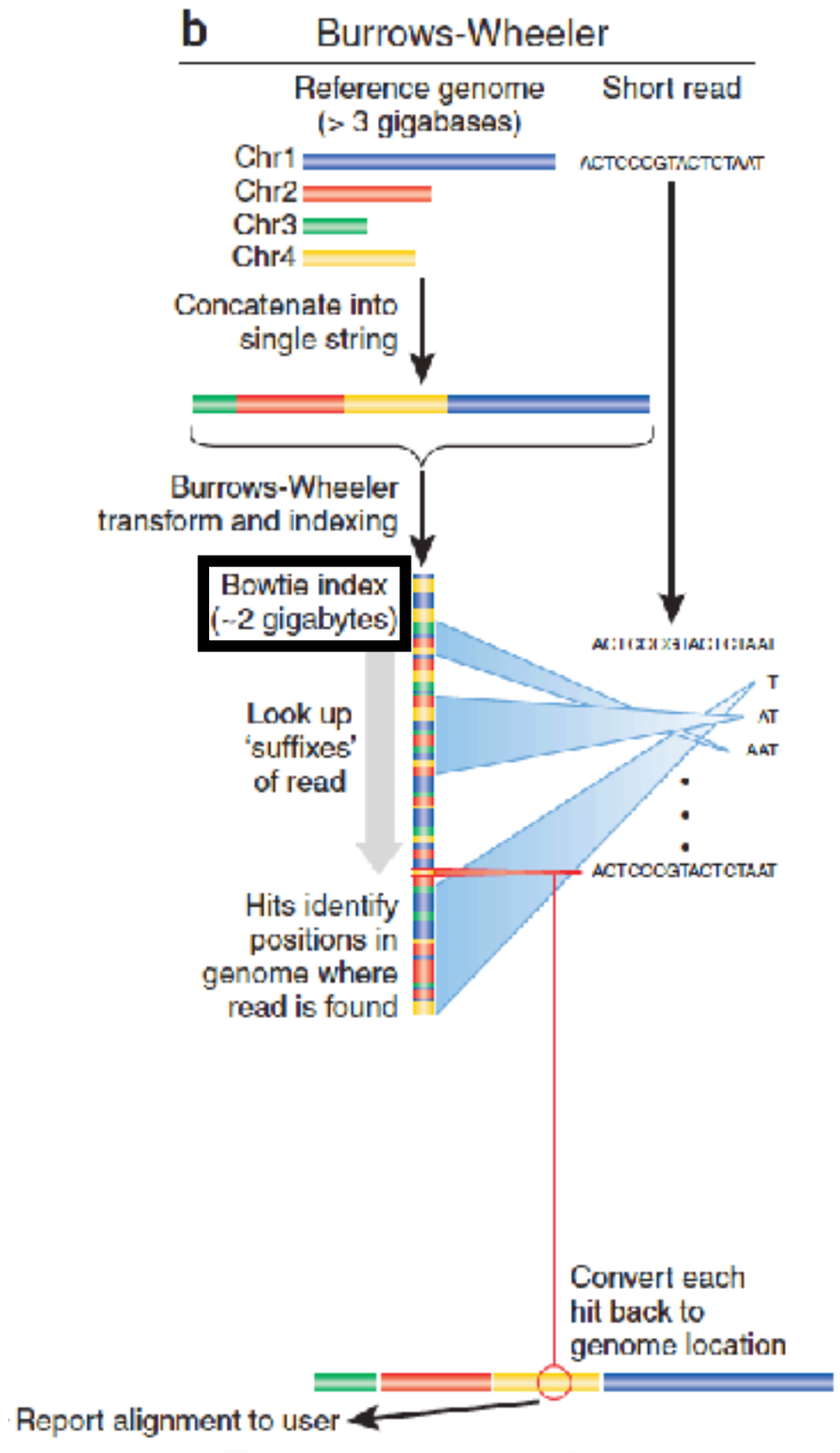
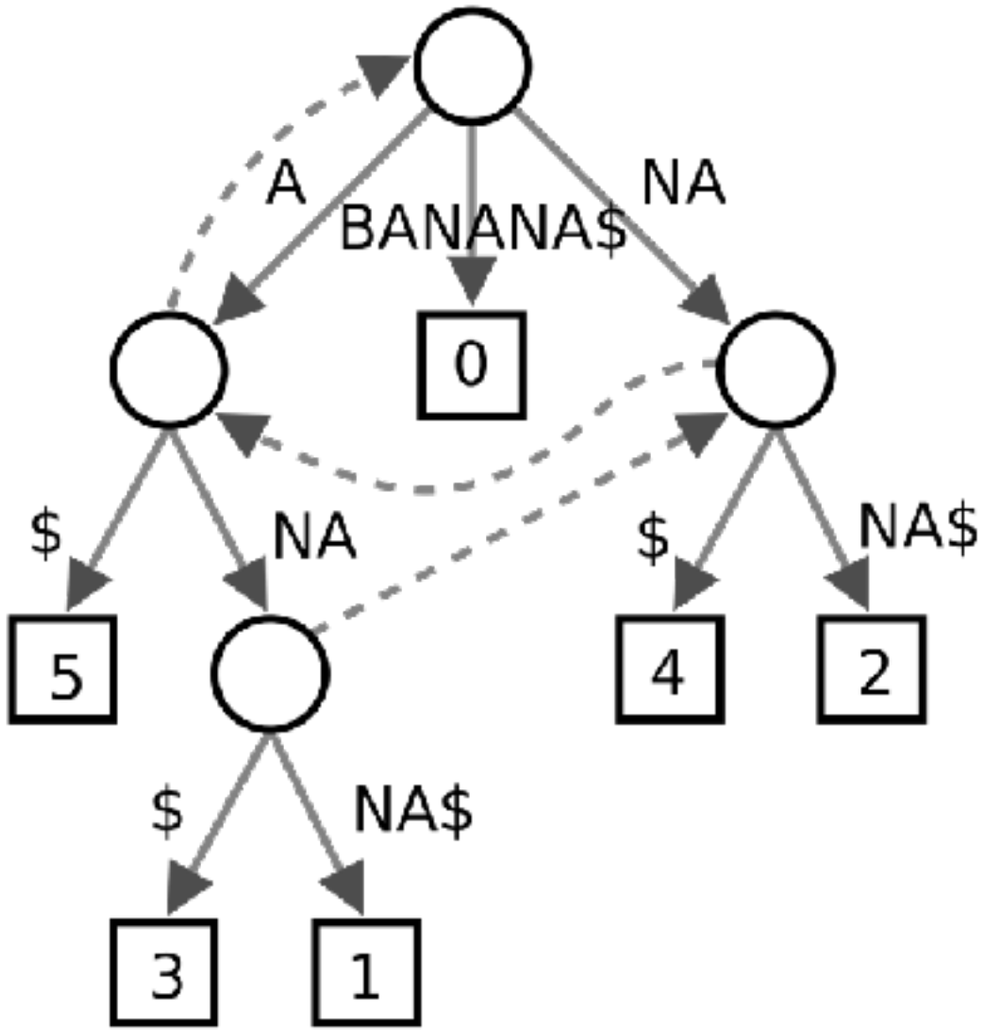


Figure modified from Trapnell & Salzberg 2009

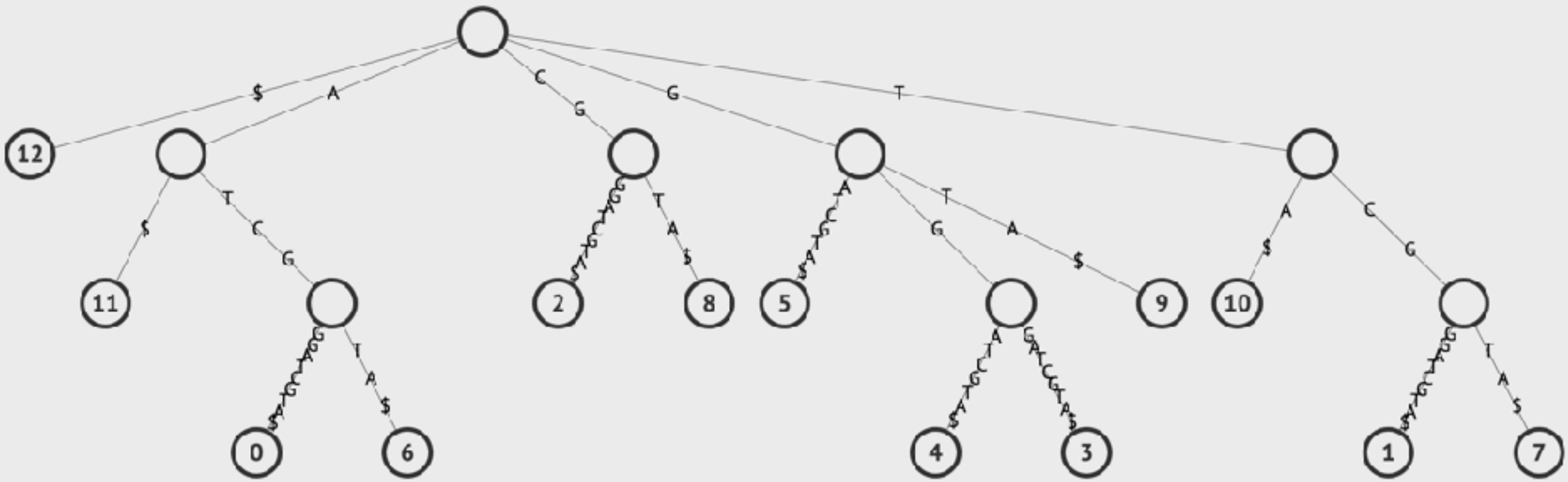
Suffix-Trie

A data structure that contains all suffixes and their locations in the text



Suffix trie for the sequence ATCGGGATCGTA

A	T	C	G	G	G	A	T	C	G	T	A	$
0	1	2	3	4	5	6	7	8	9	10	11	12

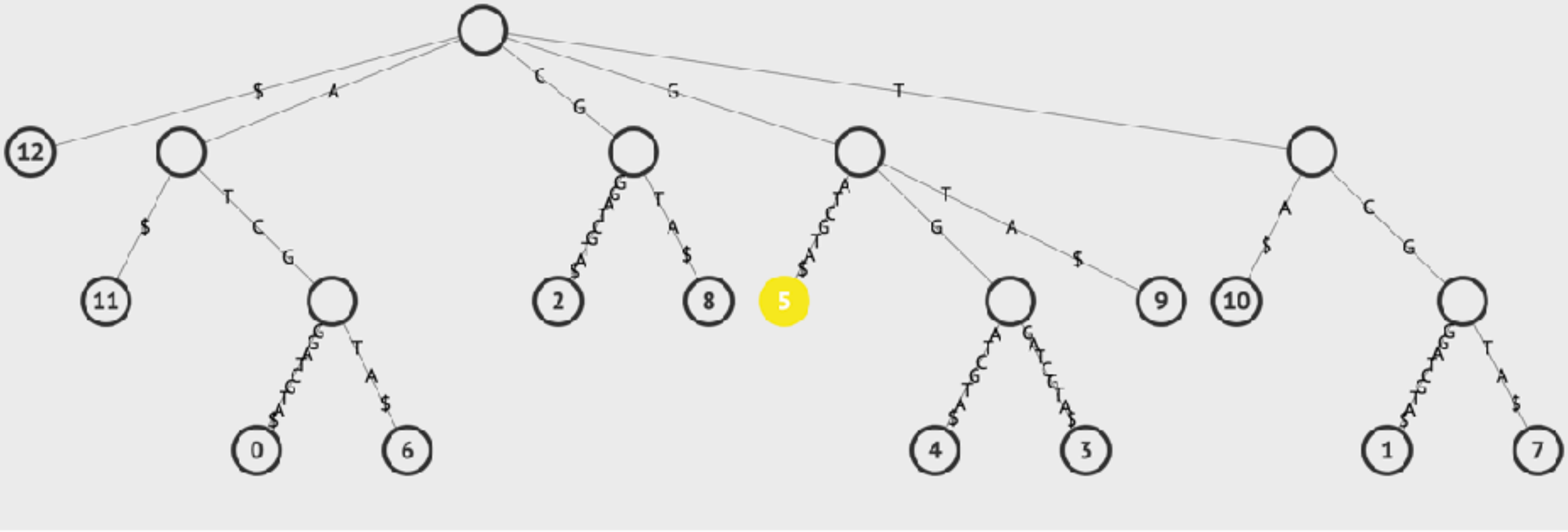


Tries built using https://visualgo.net/en/suffixtree

Suffix trie for the sequence ATCGGGATCGTA

Search for the Substring "GAT"

A	T	C	G	G	**G**	**A**	**T**	C	G	T	A	$
0	1	2	3	4	**5**	**6**	**7**	8	9	10	11	12



Tries built using https://visualgo.net/en/suffixtree

Suffix-Prefix Trie

A family of methods which uses a Trie structure to search a reference sequence (e.g. Bowtie, BWA, SOAP2)

Trie – data structure which stores the suffixes (i.e. ends of a sequence)

Key advantage over hashed algorithms:

- Alignment of multiple copies of an identical sequence in the reference only needs to be done once

- Bowtie uses something called an *FM-Index* to store Tries and drastically reduces memory requirements (e.g. Human genome can be stored in 2Gb of RAM)

- Burrows Wheeler Transform to perform fast lookups, replacing the hash

Burrows-Wheeler Algorithm

- Encodes data so that it is easier to compress
- Can be reversed to recover the original word

Transformation				
Input	All Rotations	Sorting All Rows in Alphabetical Order by their first letters	Taking Last Column	Output Last Column
^BANANA|	^BANANA| |^BANANA A|^BANAN NA|^BANA ANA|^BAN NANA|^BA ANANA|^B BANANA|^	ANANA|^B ANA|^BAN A|^BANAN BANANA|^ NANA|^BA NA|^BANA ^BANANA| |^BANANA	ANANA|^B ANA|^BAN A|^BANAN BANANA|^ NANA|^BA NA|^BANA ^BANANA| |^BANANA	BNN^AA|A

Suffix-Prefix Trie

Less sensitive for sequences that are more different from the reference so problems can arise with:

- Sequencing errors
- Query - Reference differences (i.e. divergence)

Comparison

Table 1.
Benchmark of short read alignment tools

Software	Reads aligned (%)	Time (paired, s)	Time (single, s)	Memory usage (GB)
SOAP2	93.6	828	478	5.4
SOAP	93.8	19 234	14 328	14.7
MAQ	93.2	22 506	19 847	1.2
Bowtie	91.7	–	405	2.3

Suffix

Hash

Hash

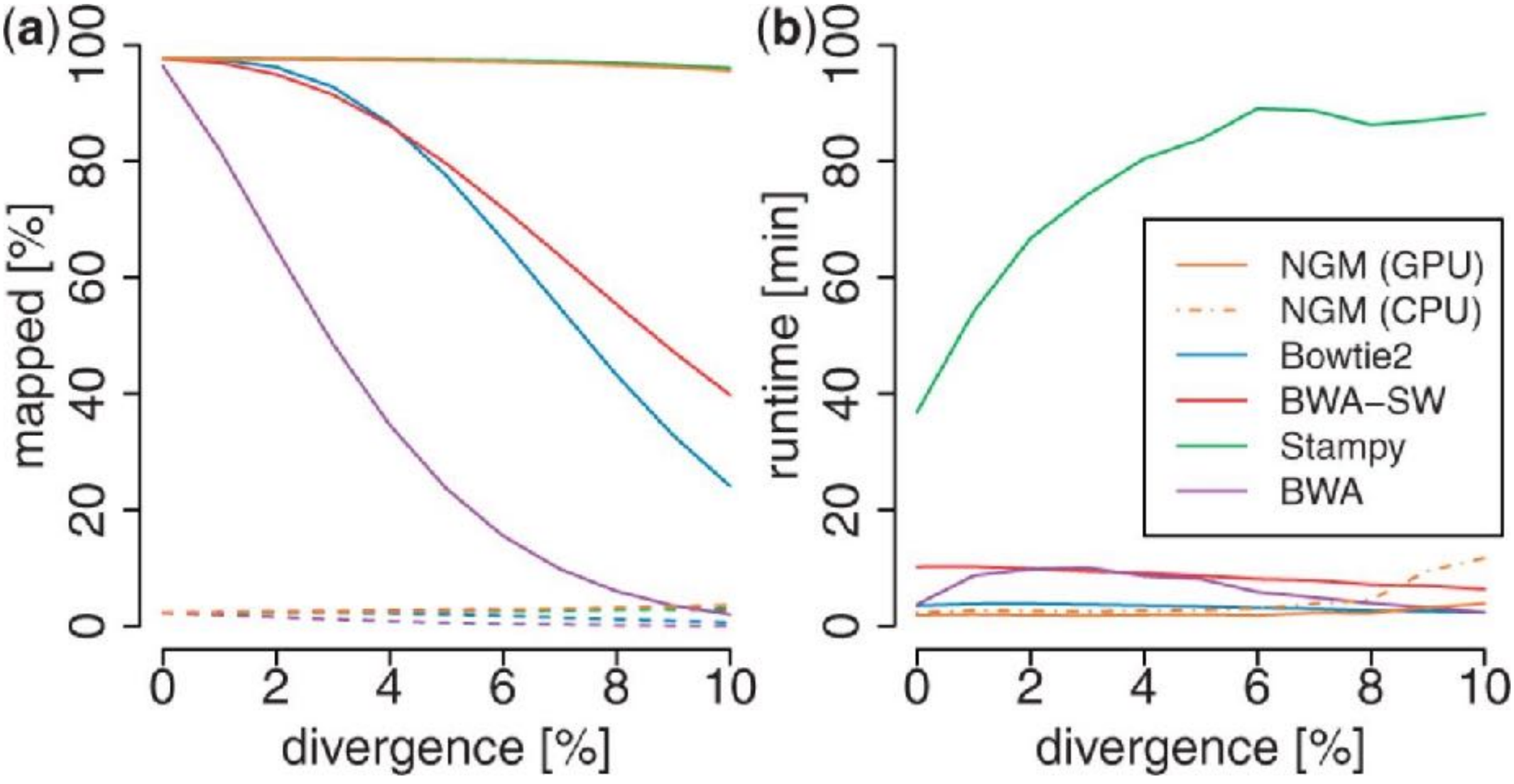
Suffix

Table from
Li et al Bioinformatics. 2009

Popular short read aligners

Program	Algorithm	Speed	Accuracy for divergent sequences
Bowtie2	Suffix/Prefix	Very fast	Low
BWA	Suffix/Prefix	Fast	Medium
Stampy	Hashing ref	Slow	High
Soap2	Suffix/Prefix	Fast	Low
Novoalign	Hashing ref	Slow	High
NextGenMap	Hashing ref	Med	High

Alignment stats



*From NextGenMap paper

Long read alignment

Long reads might contain structural variation that makes it hard to form a linear alignment

For example, a read containing a large inversion would contain 3 linear alignments

Add to that, that long read technologies have v. high error rates

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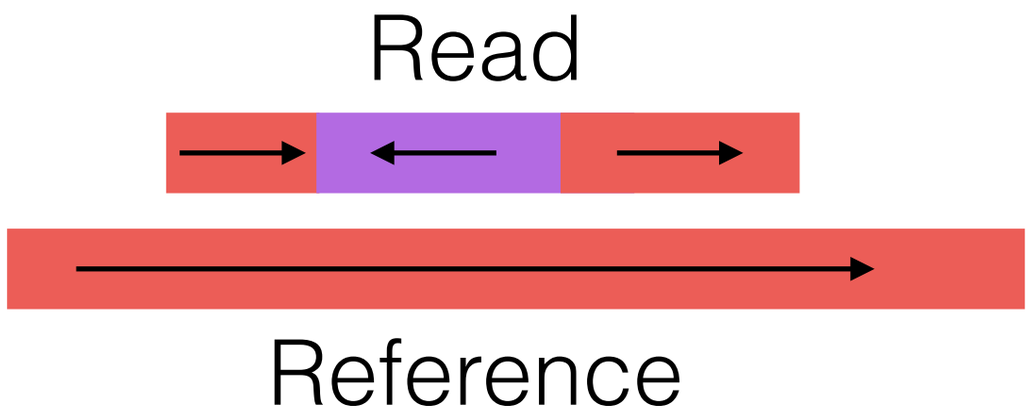
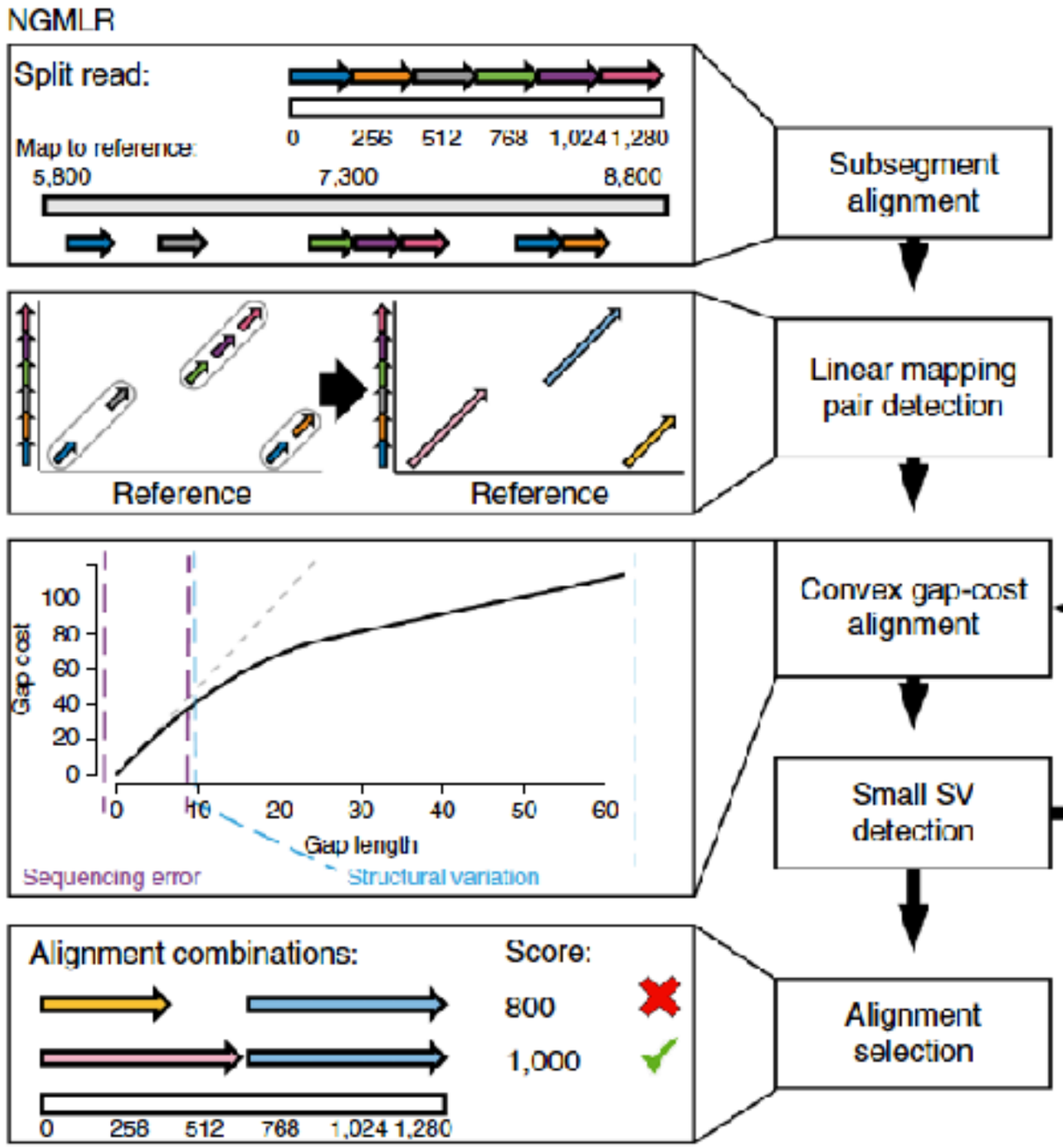


Long read alignment with NGMLR

Find exact matches between read fragment and reference

Look for chains of matches

Use local alignment of read to best reference region.



A similar approach is taken by minimap2

From NGMLR paper - Sedlazeck et al 2018 Nat. Methods

Long read alignment

Longer reads have more information, but potentially more error.

Example: The **NGMLR** software uses k-mers to map seeds then uses the Smith-Waterman algorithm for exact placements

Example: The **minimap2** software uses a hashed-seed extend approach, but a subset of all possible seeds are used. Seed matches are then chained together

Other programs:

- KART, BWA-MEM, BLASR

Alignment choice

- Speed needed?
- How divergent is sequence from reference? Same species or relative?
- How much variation in your samples?
- Genome size of reference?

SAM (BAM) format

Sequence Alignment/Map format

- Universal standard.
- Generally aligned to reference, but not necessarily
- Human-readable (SAM) and compressed (BAM) forms

Structure:

- Header: Version, sort order, reference sequences, read groups, program/processing history
- Alignment records

Keeps a track of the reference genome

And individual read records

SAM format: Header

```
@HD     VN:1.5  GO:none SO:coordinate
@SQ     SN:cp_gi_88656873       LN:151104
@SQ     SN:mt_gi_571031384      LN:300945
@SQ     SN:rDNA_gi_563582565    LN:9814
@SQ     SN:Ha1  LN:175985764
@SQ     SN:Ha2  LN:209013747
@SQ     SN:Ha3  LN:203472901
@SQ     SN:Ha4  LN:216026857
@SQ     SN:Ha5  LN:271056985
@SQ     SN:Ha6  LN:100519666
@SQ     SN:Ha7  LN:109221022
@SQ     SN:Ha8  LN:192129815
@SQ     SN:Ha9  LN:253478808
@SQ     SN:Ha10 LN:327788049
@SQ     SN:Ha11 LN:208730832
@SQ     SN:Ha12 LN:208068730
@SQ     SN:Ha13 LN:239367298
@SQ     SN:Ha14 LN:230295834
@SQ     SN:Ha15 LN:202246870
@SQ     SN:Ha16 LN:226777971
@SQ     SN:Ha17 LN:267415242
@SQ     SN:Ha0_73Ns     LN:359367108
@RG     ID:HI.2034.006.Index_18.W70_NHK_2013_5  LB:Anomalus     PL:ILLUMINA     SM:HI.2034.006.Index_18.W70_NHK_2013_5  PU:Anomalus
@PG     ID:ngm  PN:ngm  CL:" --affine 0 --argos_min_score 0 --bam 1 --block_multiplier 2 --bs_cutoff 6 --bs_mapping 0 --cpu_threads 11 --dualstrand 1
@PG     ID:ngm.1        PN:ngm  CL:" --affine 0 --argos_min_score 0 --bam 1 --block_multiplier 2 --bs_cutoff 6 --bs_mapping 0 --cpu_threads 11 --
@PG     ID:ngm.2        PN:ngm  CL:" --affine 0 --argos_min_score 0 --bam 1 --block_multiplier 2 --bs_cutoff 6 --bs_mapping 0 --cpu_threads 11 --
```

SAM format: Header

```
@HD     VN:1.5  GO:none SO:coordinate
@SQ     SN:cp_gi_88656873       LN:151104
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@SQ     SN:Ha13 LN:239367298
@SQ     SN:Ha14 LN:230295834
@SQ     SN:Ha15 LN:202246870
@SQ     SN:Ha16 LN:226777971
@SQ     SN:Ha17 LN:267415242
@SQ     SN:Ha0_73Ns     LN:359367108
@RG     ID:HI.2034.006.Index_18.W70_NHK_2013_5  LB:Anomalus     PL:ILLUMINA     SM:HI.2034.006.Index_18.W70_NHK_2013_5  PU:Anomalus
@PG     ID:ngm  PN:ngm  CL:" --affine 0 --argos_min_score 0 --bam 1 --block_multiplier 2 --bs_cutoff 6 --bs_mapping 0 --cpu_threads 11 --dualstrand 1
@PG     ID:ngm.1        PN:ngm  CL:" --affine 0 --argos_min_score 0 --bam 1 --block_multiplier 2 --bs_cutoff 6 --bs_mapping 0 --cpu_threads 11 --
@PG     ID:ngm.2        PN:ngm  CL:" --affine 0 --argos_min_score 0 --bam 1 --block_multiplier 2 --bs_cutoff 6 --bs_mapping 0 --cpu_threads 11 --
```

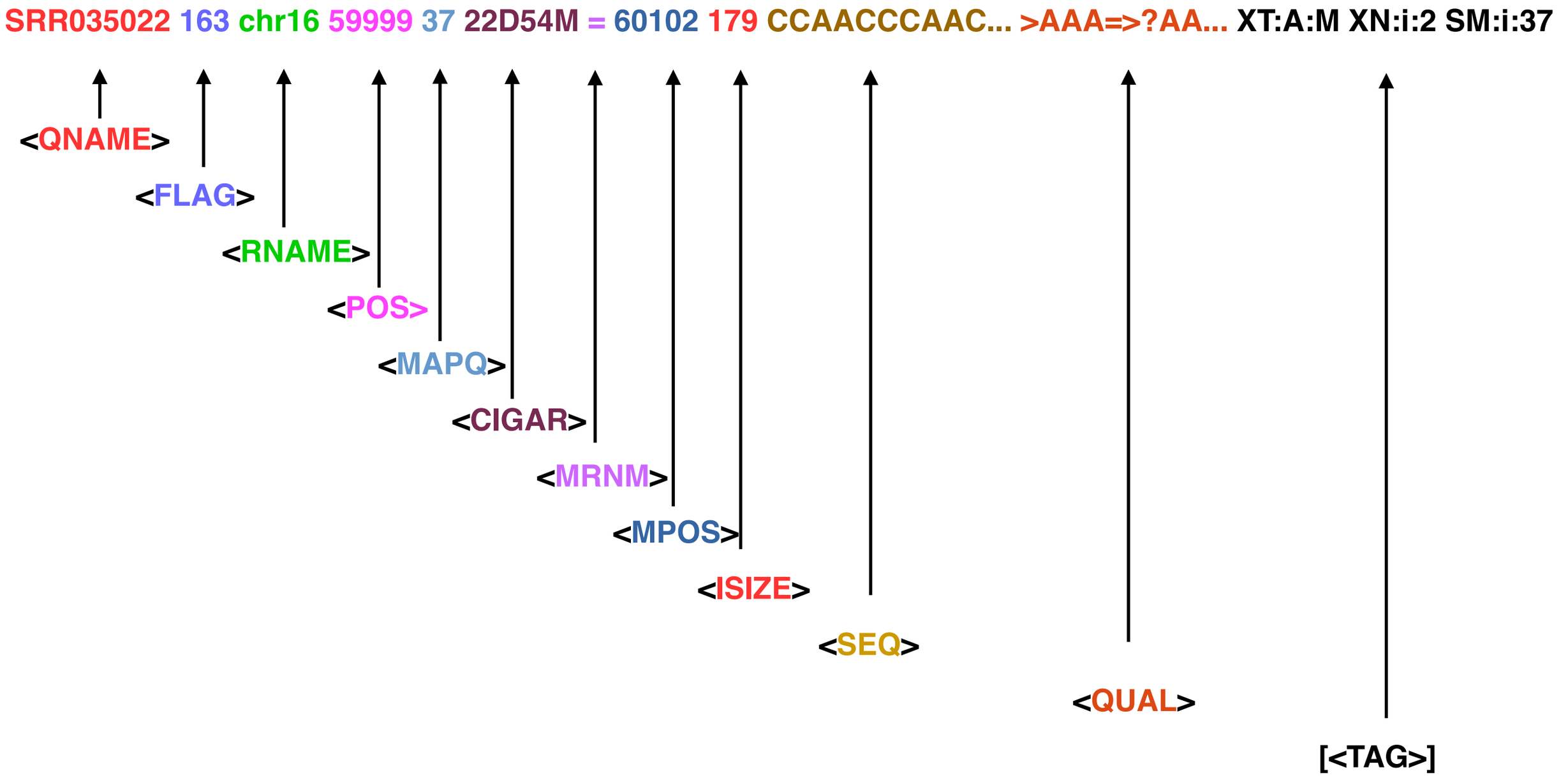
Sort order - in this case coordinate based

Reference sequence name (SN) and length (LN)
e.g. Chromosome Ha7, which is 109,221,022bp long

Read group (@RG) information

Program (@PG) information - what you used to map the reads

SAM format: Read lines



SAM format: Read lines

SRR035022 163 chr16 59999 37 22D54M = 60102 179 CCAACCCAAC... >AAA=>?AA... XT:A:M XN:i:2 SM:i:37

<QNAME> Query name - i.e. the name of the read

<FLAG> A combination of bitwise flags that indicate properties of the alignment (complement, strand etc.)

<RNAME> Reference sequence name

<POS> Position in the reference (1-based)

<MAPQ> Mapping quality

<CIGAR> Concise Idiosyncratic Gapped Alignment Report (CIGAR) string - tells you about gaps in the alignment

<MRNM> Read-mate reference sequence

<MPOS> Read-mate position in the reference

<ISIZE> Insert size

<SEQ> The segment's sequence

<QUAL> An ASCII sequence containing quality information for each base in the sequence

[<TAG>] These are optional tags that get added and contain user specified data (For example, SM is the mapping quality of this sequence only - ignoring the read mate)

SAM format: Read lines

SRR035022 163 chr16 59999 37 22D54M = 60102 179 CCAACCCAAC... >AAA=>?AA... XT:A:M XN:i:2 SM:i:37

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<M

<C ent

<M

<M

<IS

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For an explanation of bitwise flags:

https://broadinstitute.github.io/picard/explain-flags.html

Mapping Quality

- MapQ = Qs = -10 $\log_{10}(P)$
- P = probability that this mapping is NOT the correct one
- MapQ = 0 = equally likely to map somewhere else
- Different programs use different formulas for P
- A value of 255 indicates that the information is not available

Tutorial:

Work through the tutorial associated with this session

Further Reading

A concise read:

Trapnell, C., & Salzberg, S. L. (2009). How to map billions of short reads onto genomes. *Nature biotechnology*, *27*(5), 455-457.

A more in depth read:

Reinert, K., Langmead, B., Weese, D., & Evers, D. J. (2015). Alignment of next-generation sequencing reads. *Annual review of genomics and human genetics*, *16*, 133-151.

All available on the GitHub

Seed-extend algorithm