TOPIC 4: Sequence alignment

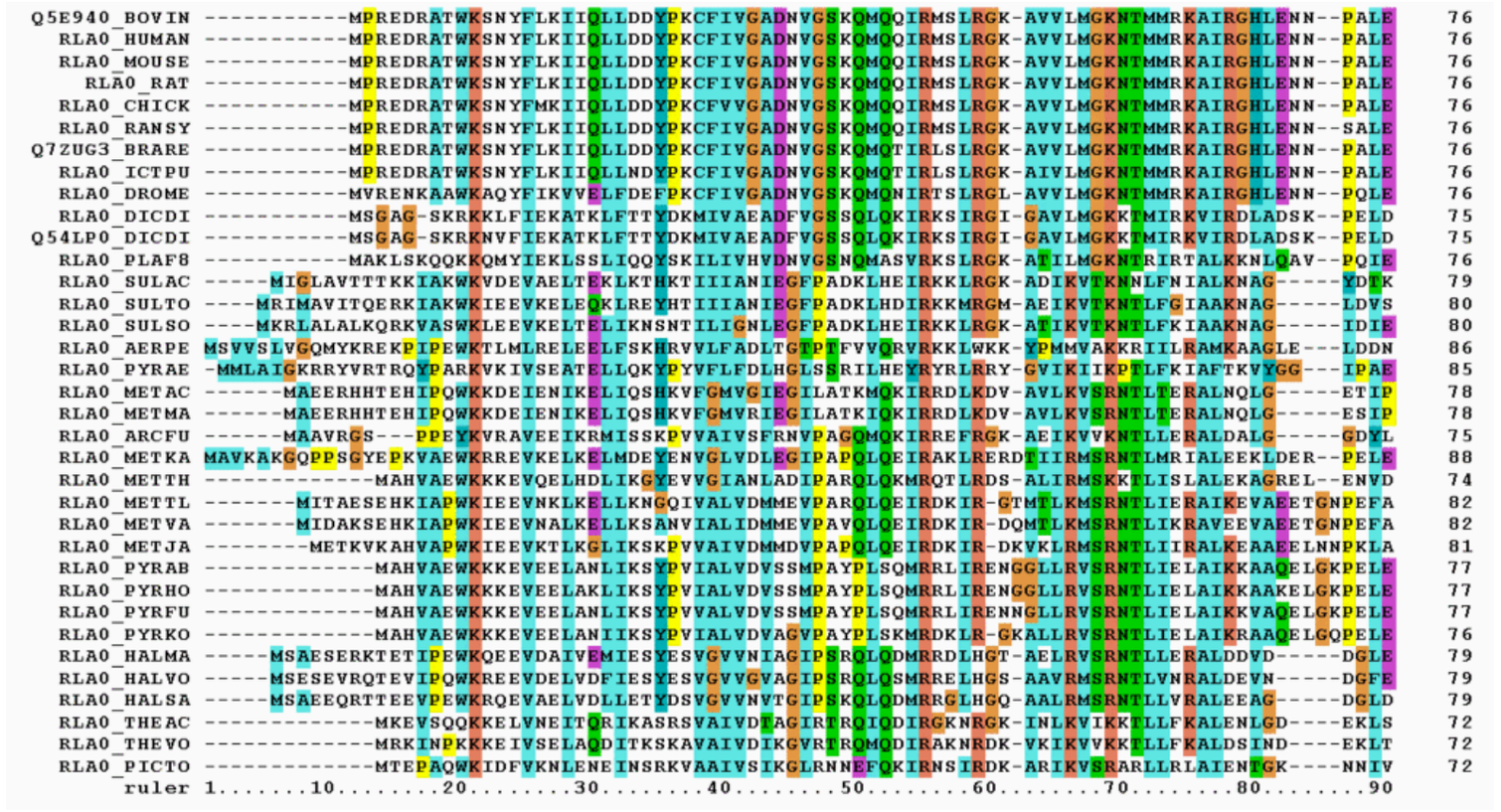
Biol 525D - Bioinformatics for Evolutionary Biology 2020

Learning Goals

- Be able to define the two main methods of alignment
- Understand the two main algorithms for NGS alignment, including strengths and weaknesses
- Be able to read SAM format

Sequence alignment

Sequence alignment is a way of arranging the sequences of DNA, RNA, or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences.



A multiple alignment of protein sequences

From Wikipedia

Pairwise alignment

Alignment of two sequences is a relatively straightforward computational problem, but…

- there are many possible alignments

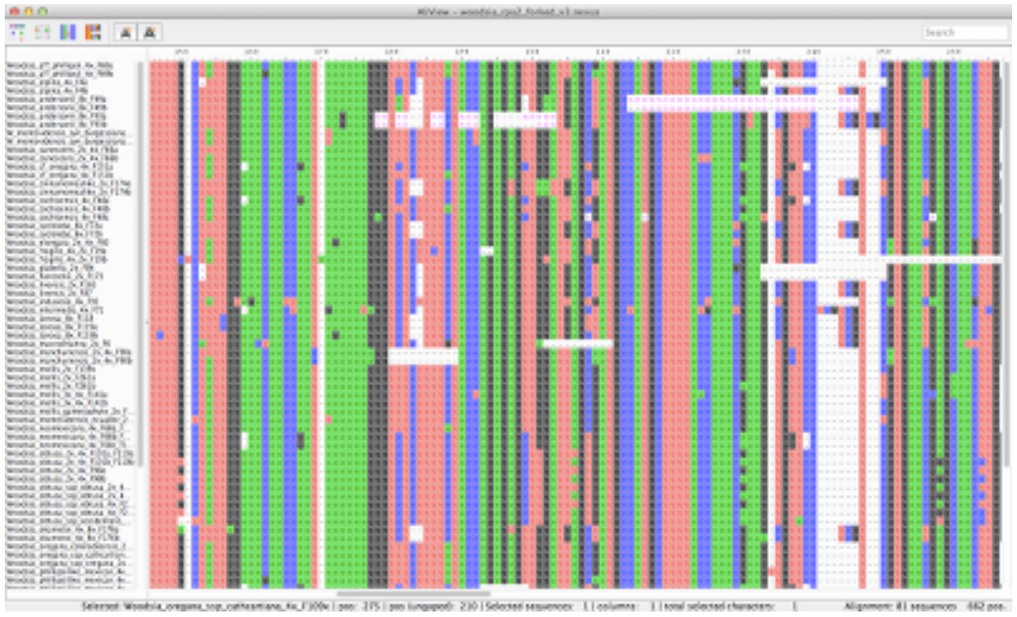
- there can be a very large reference

NOTE: Two sequences can always be aligned and there can be more than one optimal solution

Methods of alignment

By hand

- Can be accurate, but a bit fishy



Mathematical approach

- Dynamic programming (slow, but optimal)

Heuristic methods (fast, but approximate)

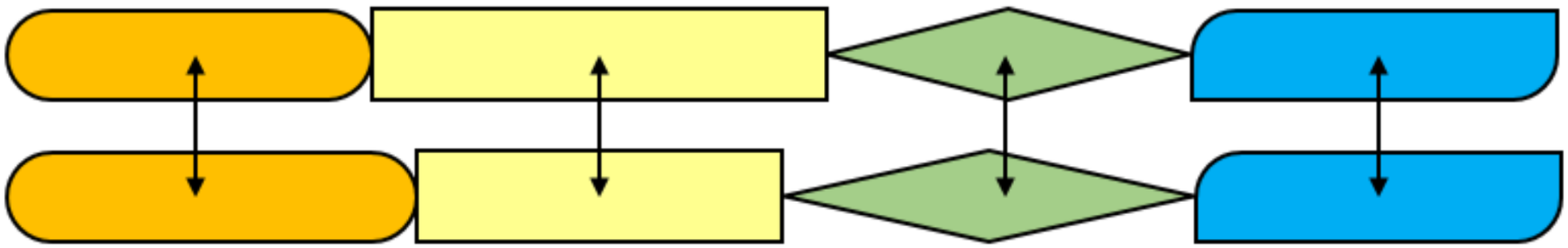
- BLAST, short read aligners

Dynamic programming

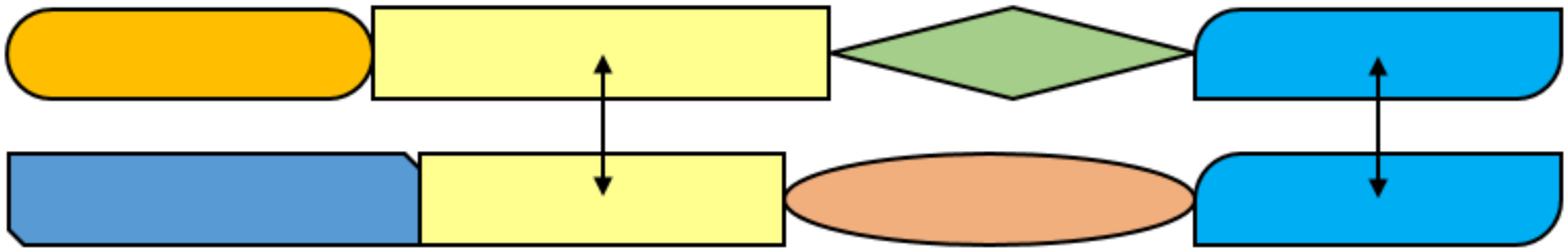
Dynamic programming is a general programming technique

It structures a large search space into a succession of stages

- The initial stage contains trivial solutions to sub-problems
- Each partial solution in a later stage can be calculated by recurring a fixed number of partial solutions in an earlier stage
- The final stage contains the overall solution



Global Alignment



Local Alignment

https://upload.wikimedia.org/wikipedia/commons/0/03/Alignment-Comparison-En.png

Global vs Local alignments

Global alignment algorithms start at the beginning of two sequences and add gaps to each until the end of one is reached (Needleman-Wunsch algorithm).

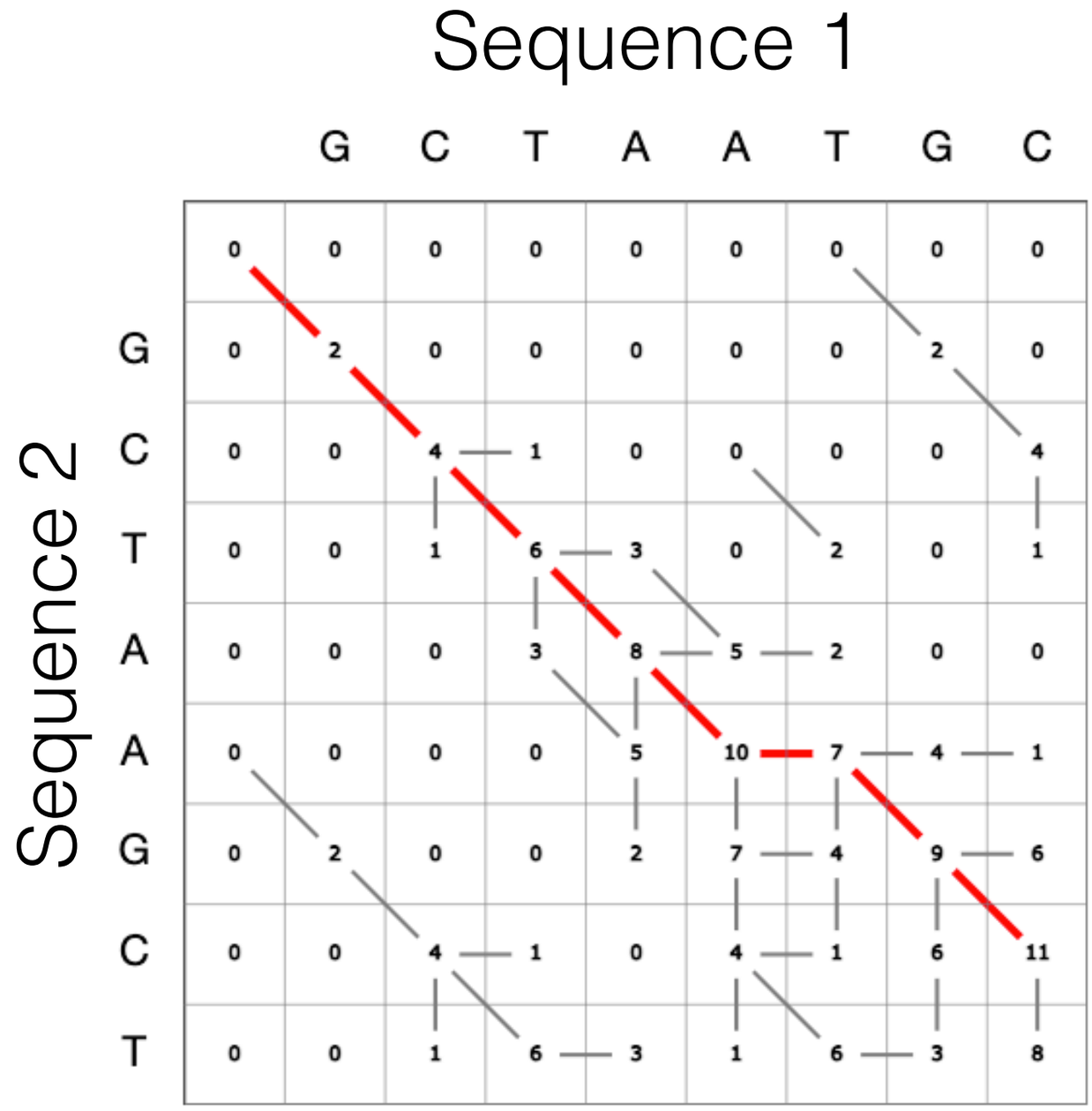
Local alignment algorithms finds the region (or regions) of highest similarity between two sequences (e.g. the Smith-Waterman algorithm).

Here's a fun demo of the two algorithms: https://gtuckerkellogg.github.io/pairwise/demo/

Basic principles of dynamic programming

There are too many comparisons to try them all so instead:

- Build alignment path matrix
- Stepwise calculation of score values
- Backtracking (evaluation of optimal path)



Scoring methods

Scoring systems:

- Each symbol pairing is assigned a numerical value, based on a symbol comparison table.
    - nucleotides
    - amino acids (PAM, BLOSUM)

Gap penalties:

- Opening: The cost of introducing a gap.
- Extension: The cost to elongate a gap.

Gap penalties

- Too little gap penalty gives nonsense non-homologous alignments.

- Gaps are common, so too high gap penalty removes real alignments.

- There are multiple gap penalty functions (e.g. constant, linear and “affine”)

- The “affine”is the most commonly used gap penalty function (e.g. BLAST and BWA use it)

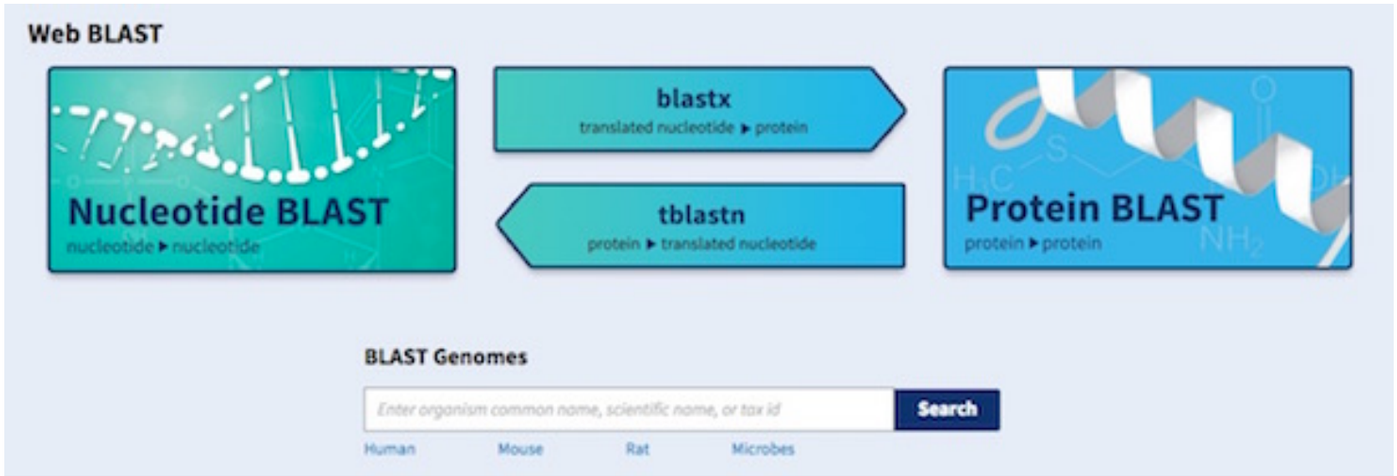
The “affine” gap penalty function

$$GP = A + BL$$

Where A is the penalty for opening a gap, B is the penalty for extending a gap and L is the length of the gap

BLAST - Best Local Alignment Search Tool

A great tool for comparing a small number of sequences against a database (e.g. NCBI BLAST)

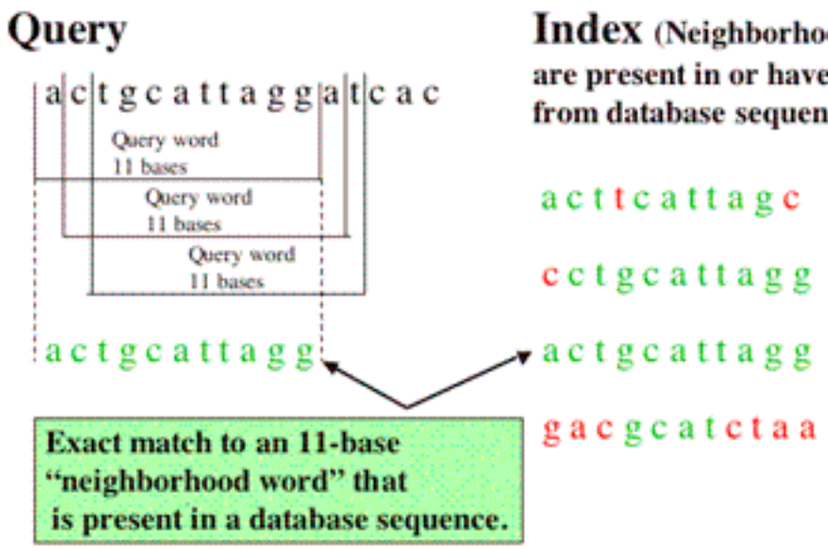


- An example of a “hashed seed-extend algorithm”

BLAST - Best Local Alignment Search Tool

Designed to identify homologous sequences.

First finds highly conserved or identical sequences which are then extended with a local alignment

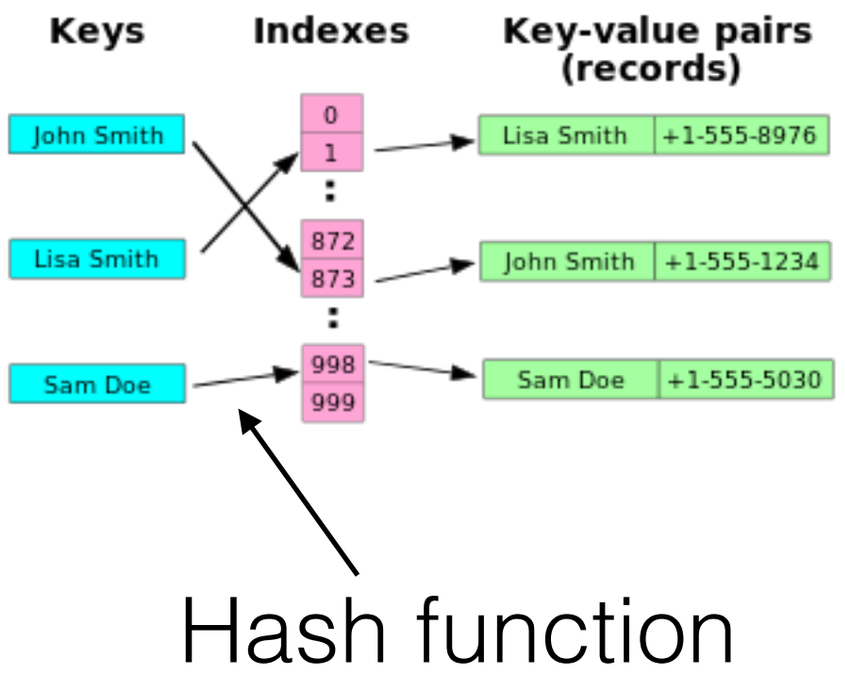


Hashed seed-extend algorithm

- A “hash” is a structure used in computer programming

- It is a way of storing information in a look-up table

- Allows efficient searching

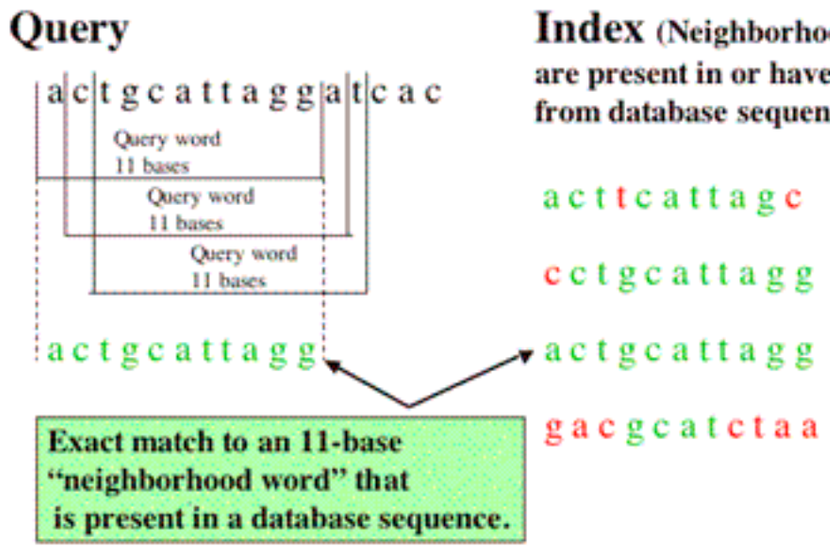


BLAST - Best Local Alignment Search Tool

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



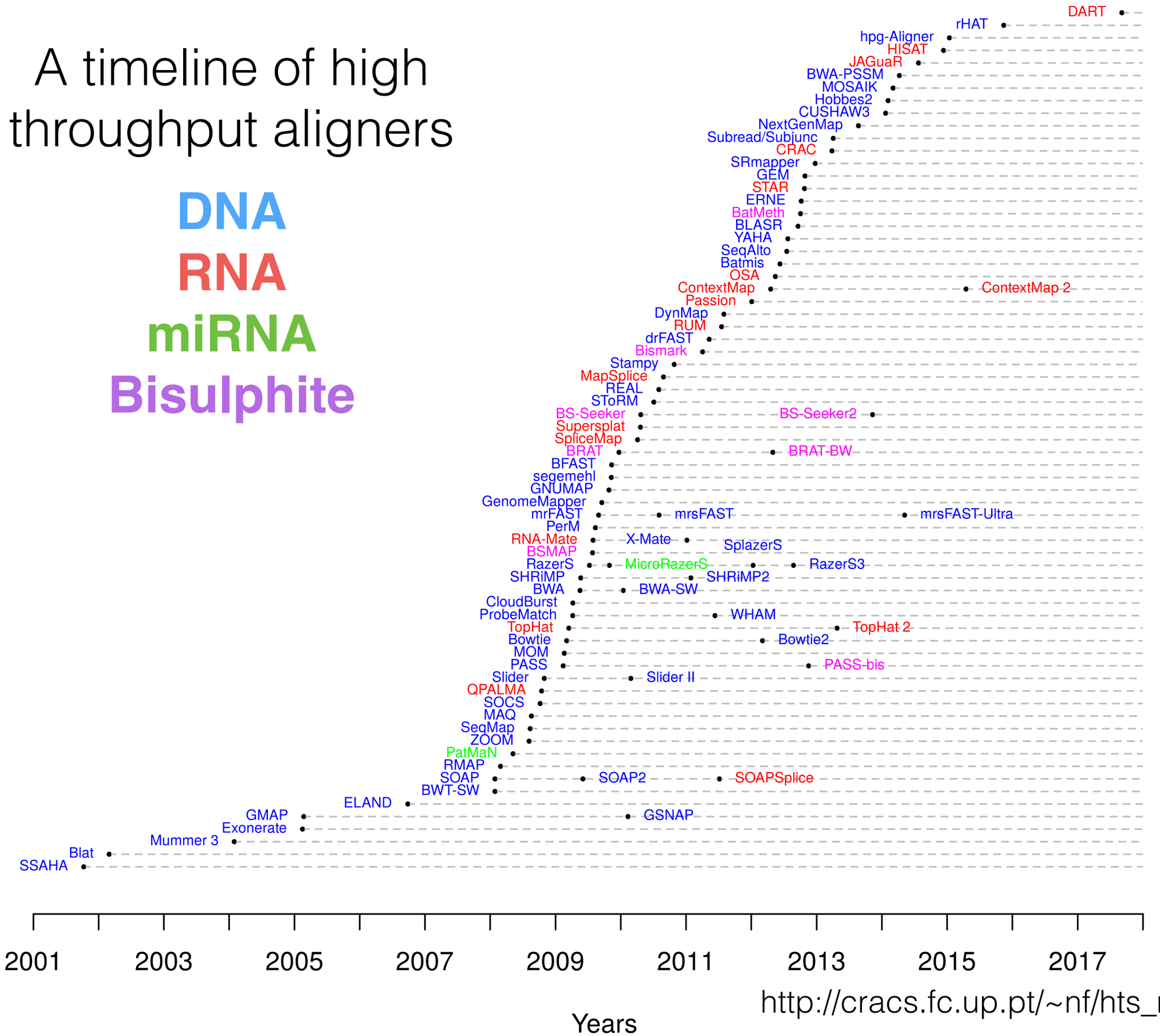
A timeline of high throughput aligners

DNA

RNA

miRNA

Bisulphite



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

Approaches to align short reads

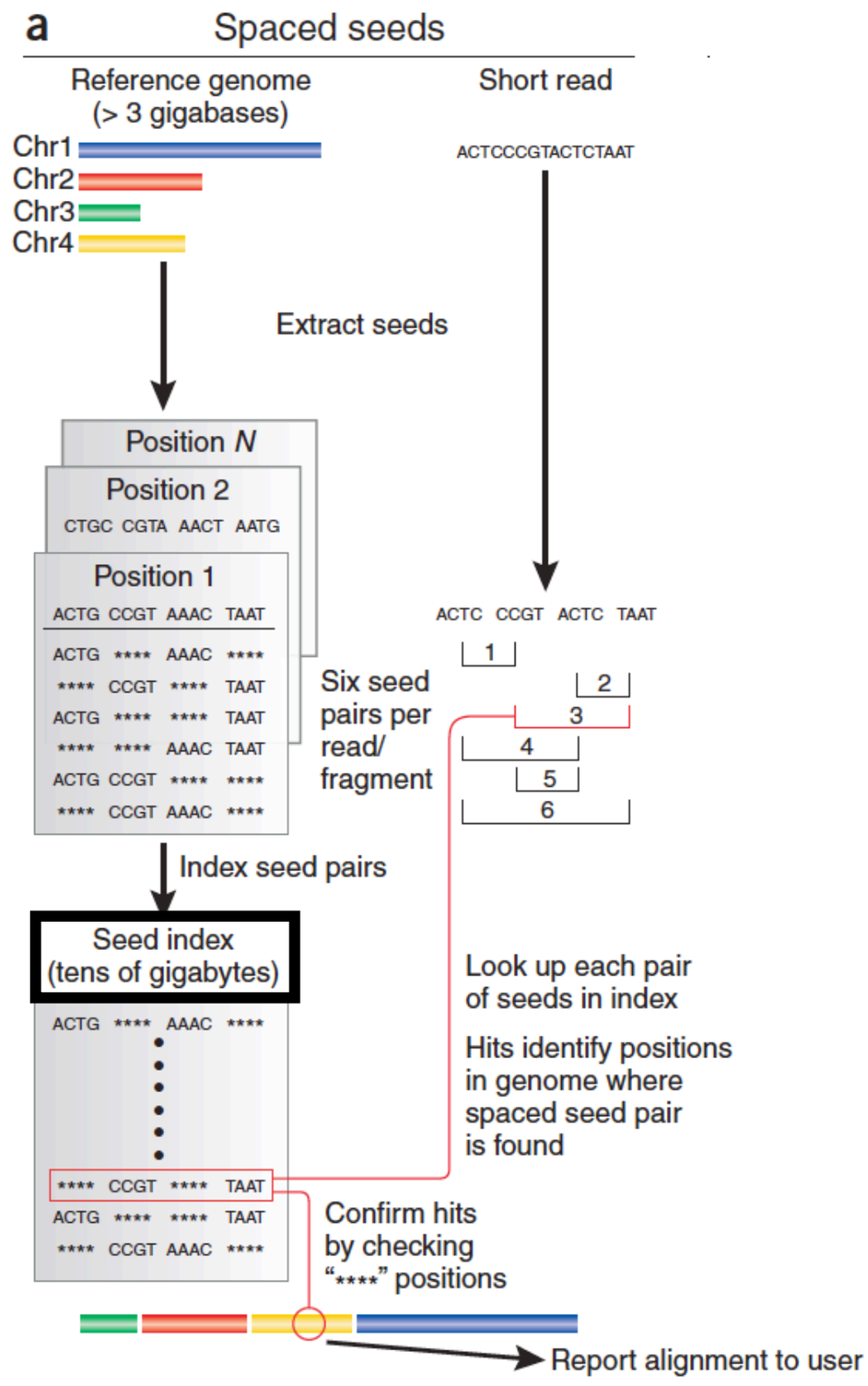


Figure modified from Trapnell & Salzberg 2009

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

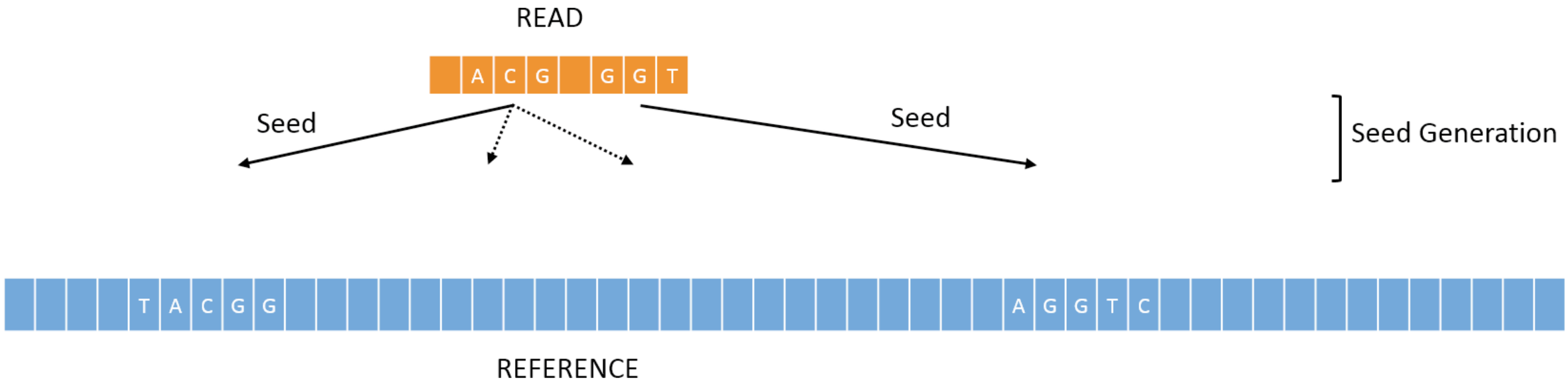
- Extend match using sensitive (but slow) Smith-Waterman algorithm

Seed-extend algorithm

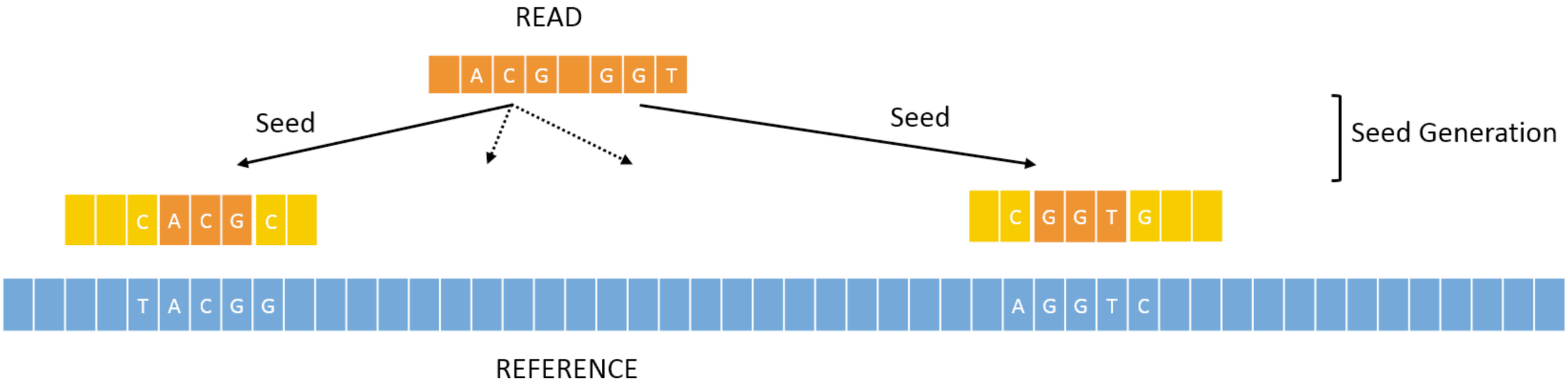
READ

REFERENCE

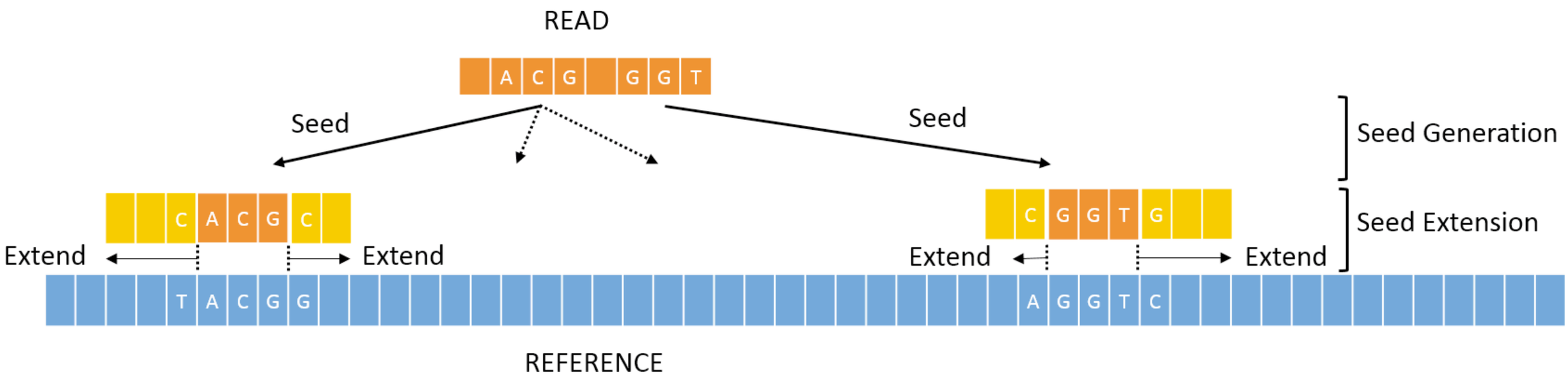
Seed-extend algorithm



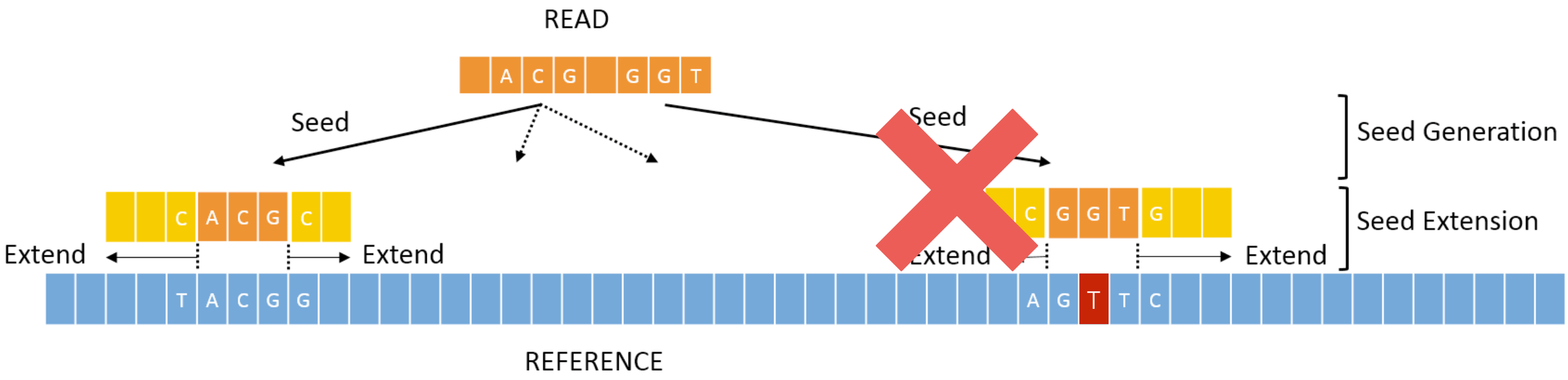
Seed-extend algorithm



Seed-extend algorithm



Seed-extend algorithm



Spaced seeds

To increase sensitivity we can used spaced-seeds:

```
111111111
GATAGCTAGCTAAT
 AGCTAGCTA
```

Consecutive seed template with **length** 9bp

Reference

Query

```
101011010011011
GATAGCTAGCTAAT
GATAGCGAGCTAAT
```

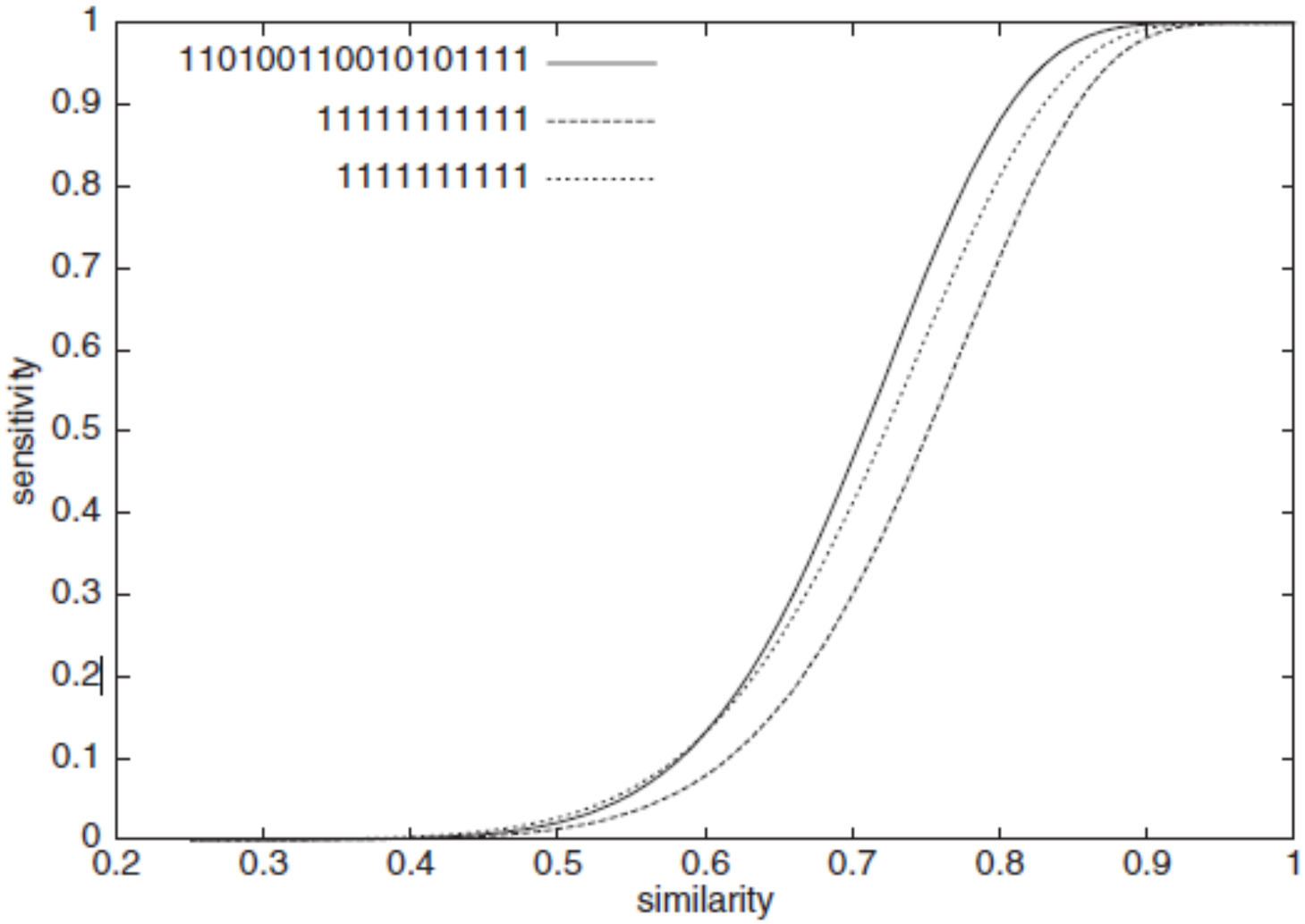
Consecutive seed template with **weight** 9bp

Reference

Query

Spaced seeds

- To increase sensitivity we can used spaced-seeds:



Approaches to align short reads

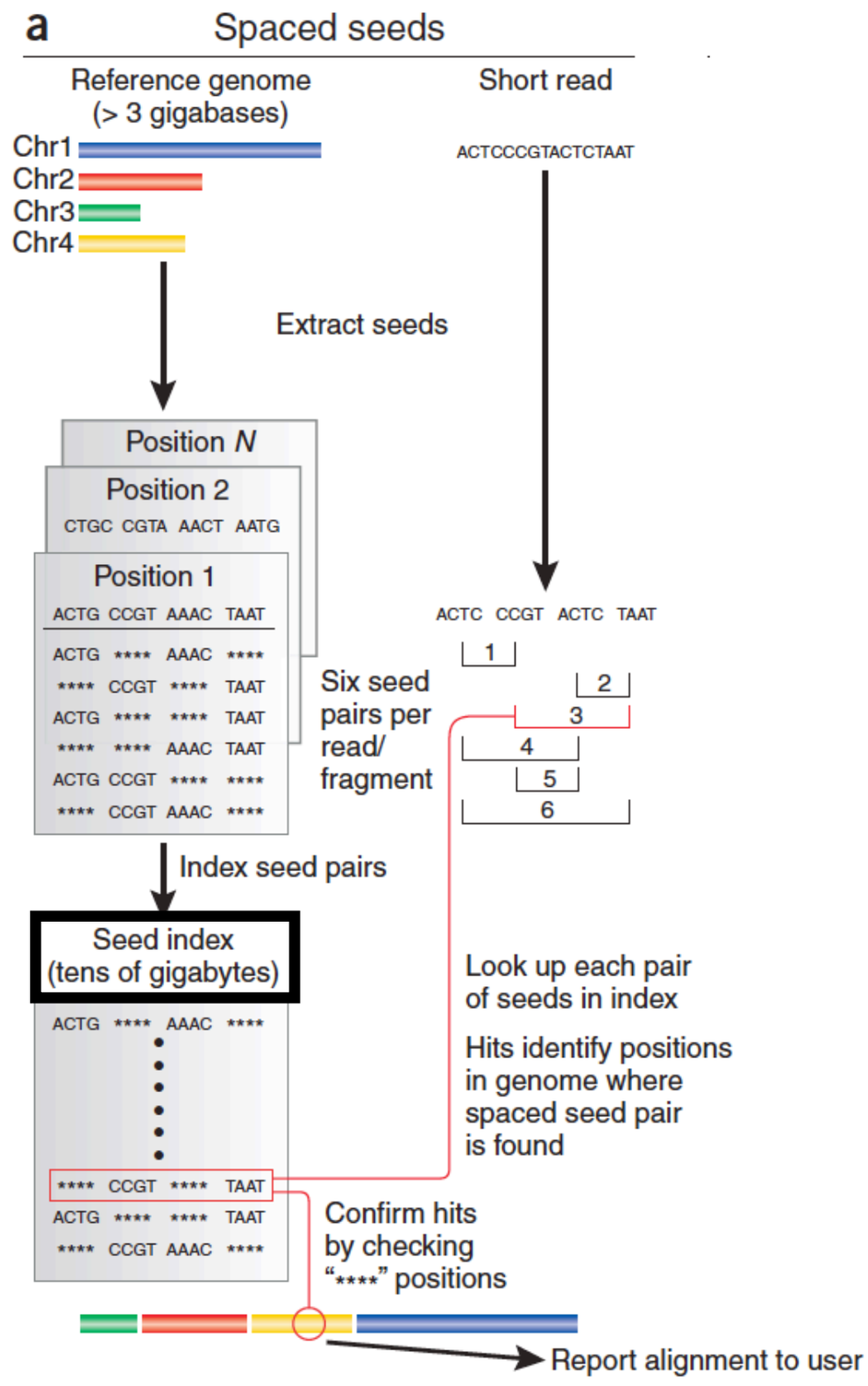


Figure modified from Trapnell & Salzberg 2009

Approaches to align short reads

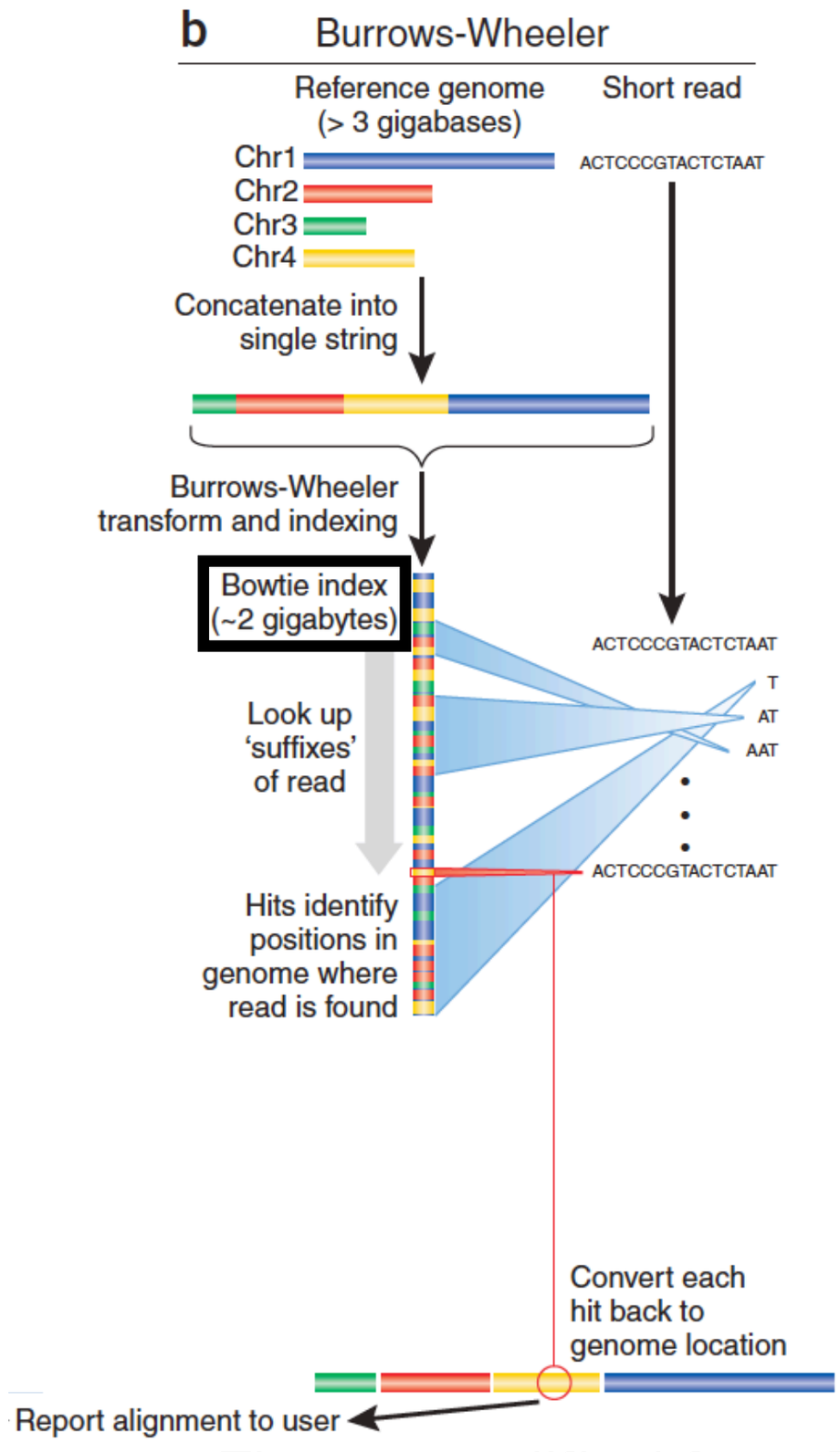
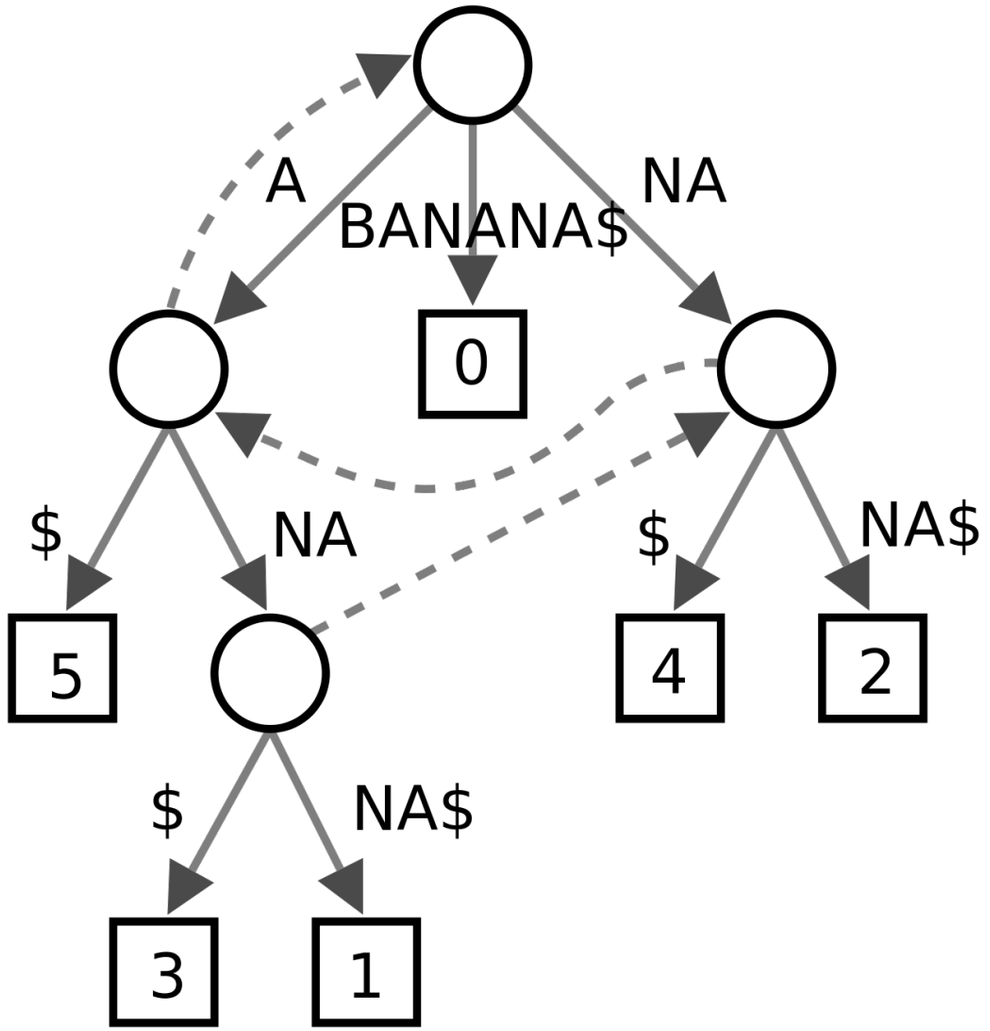


Figure modified from Trapnell & Salzberg 2009

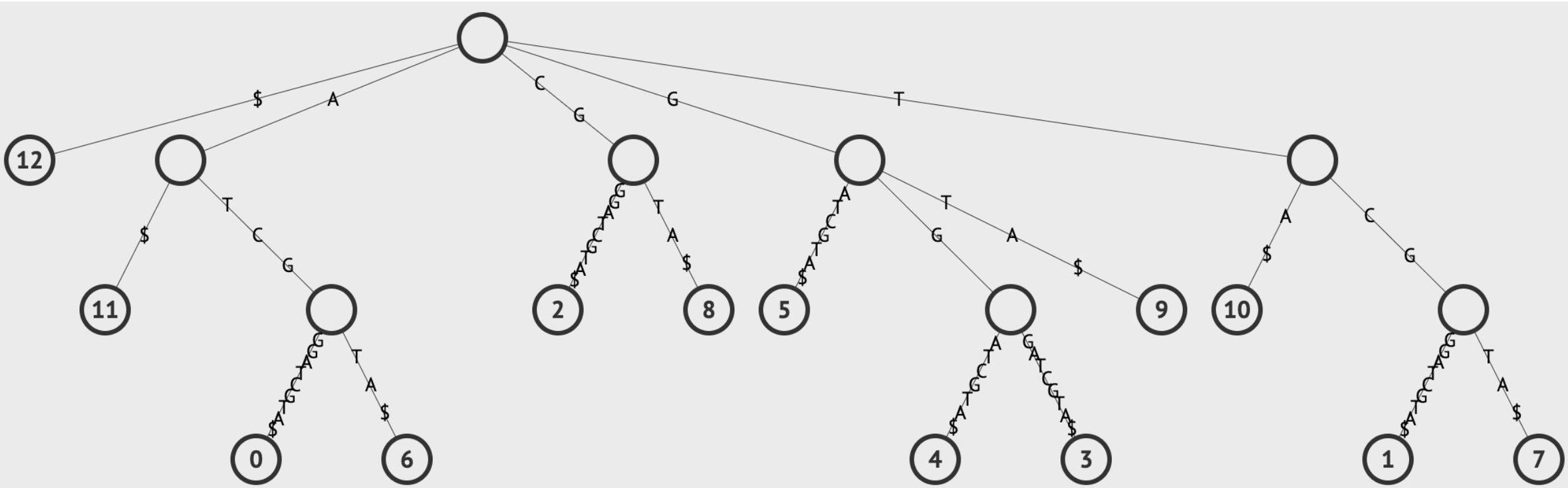
Suffix-Trie

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

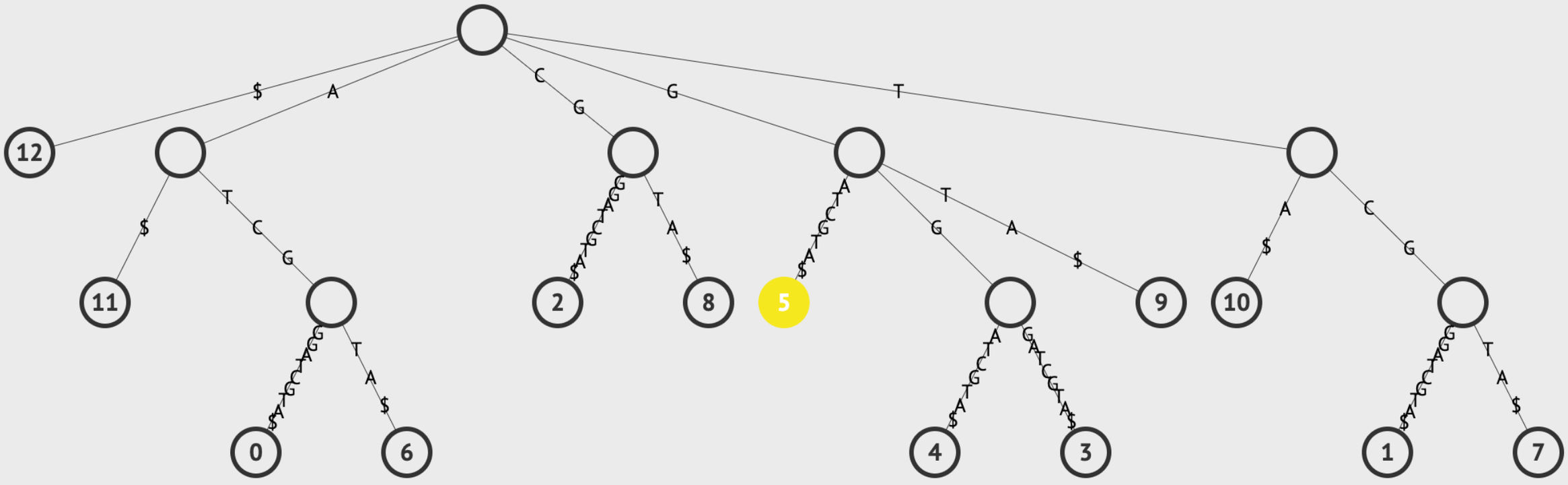


Tries built using https://visualgo.net/en/suffixtree

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
- Use of an *FM-Index* to store Trie can drastically reduce memory requirements (e.g. Human genome can be stored in 2Gb of RAM)
- Burrows Wheeler Transform to perform fast lookups

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

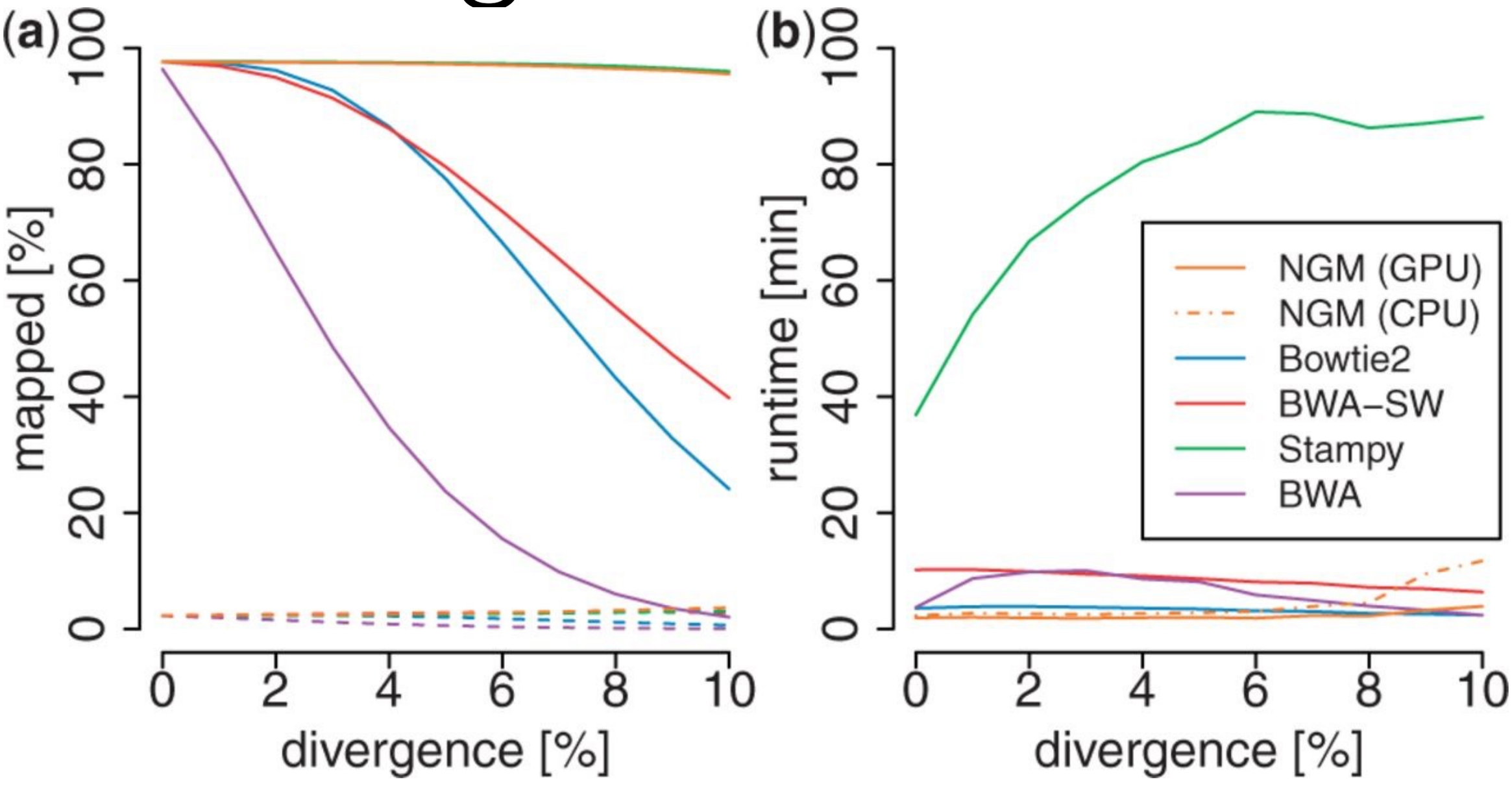
Suffix

Table from
Li et al Bioinformatics. 2009

Popular short read aligners

Program	Algorithm	Speed	Accuracy in 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

Think-Pair-Share

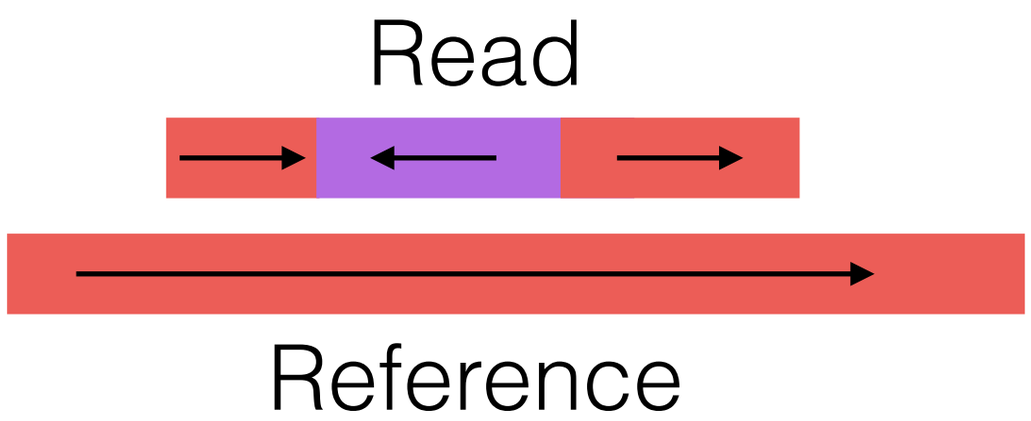
- Third generation sequencing can produce very long reads (10-50 Kbp), but are very error prone (~5-10% errors)
- Why would suffix-trie based aligners do poorly with this data?

Long read alignment with NGMLR

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

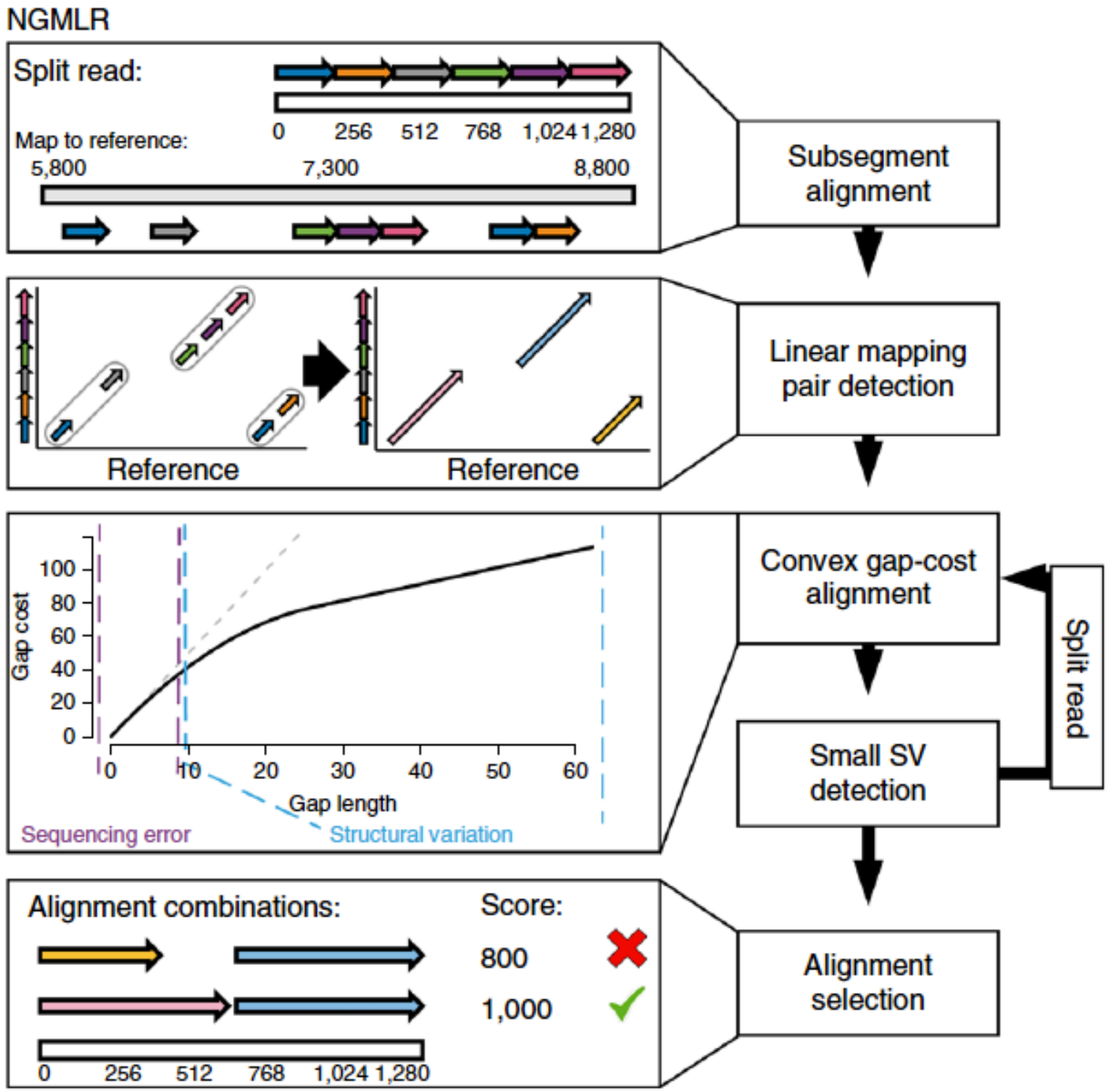


Long read alignment with NGMLR

Find exact matches between read fragment and reference

Look for chains of matches

Use local alignment of read to best reference region.



From NGMLR paper - Sedlazeck et al 2018 Nat. Methods

Long read alignment

- Longer reads have more information, but more error.

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

- Other programs:

  - KART, BWA-MEM, BLASR, minimap2

Alignment choice

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

Other considerations

PCR duplicates

Multi-mapping reads

Spliced-read mapping

PCR duplicates

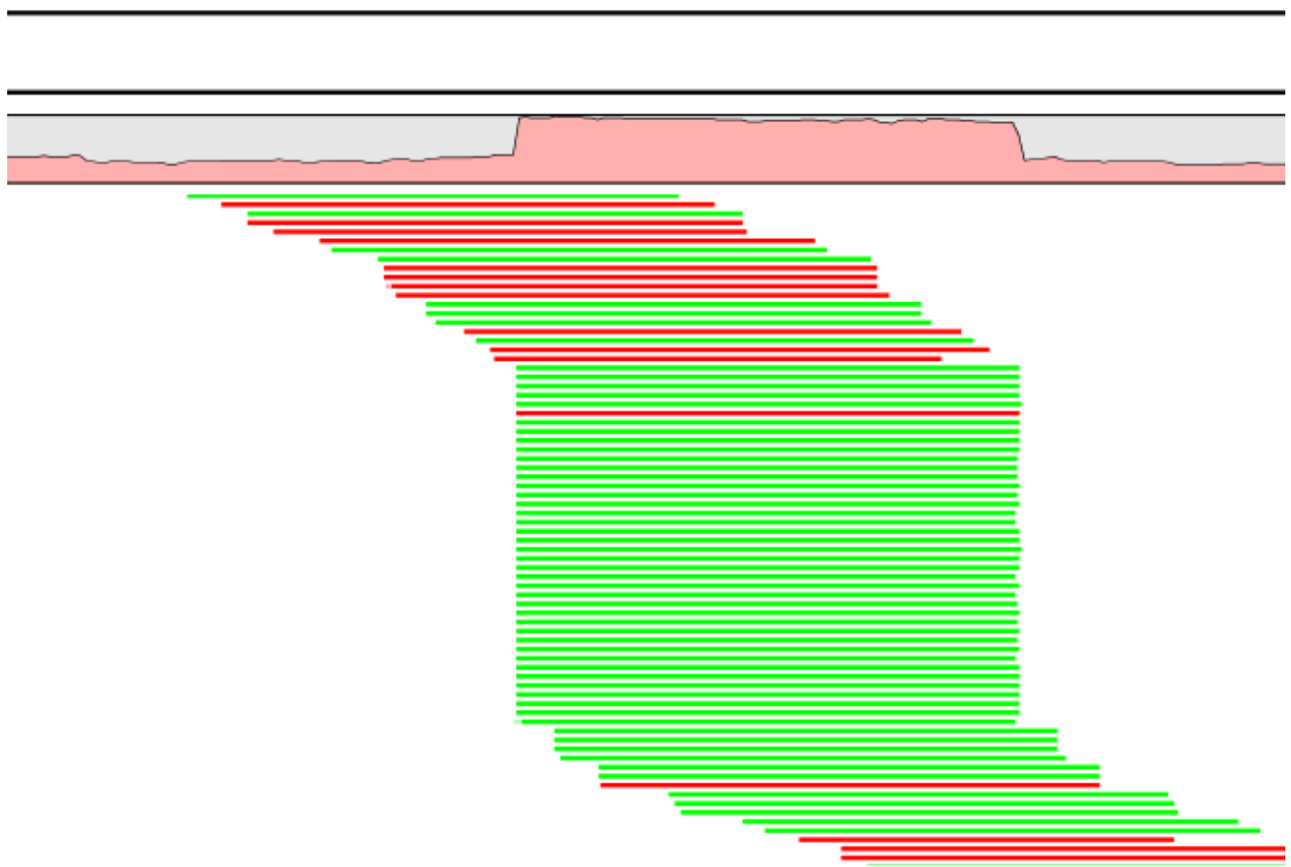
Most library preps have at least one PCR amplification step

PCR can introduce errors and then sequencing multiple copies makes it seem like a real SNP

There are standard tools for flagging or removing these duplicates based on alignment location

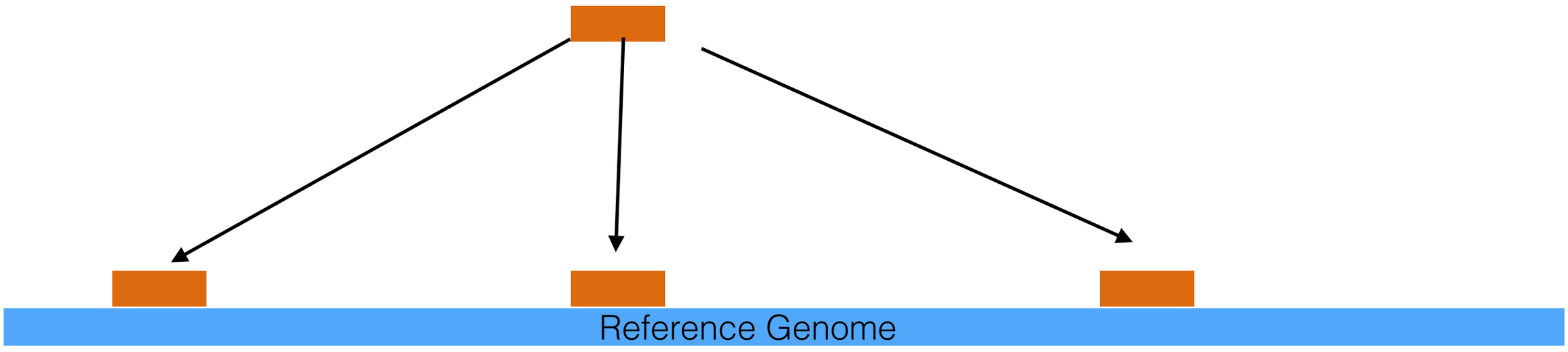
- Samples with same start and stop position are considered duplicates

- Don’t flag duplicates for GBS (set start and stop)



From Qiagen website

Multi-mapping reads

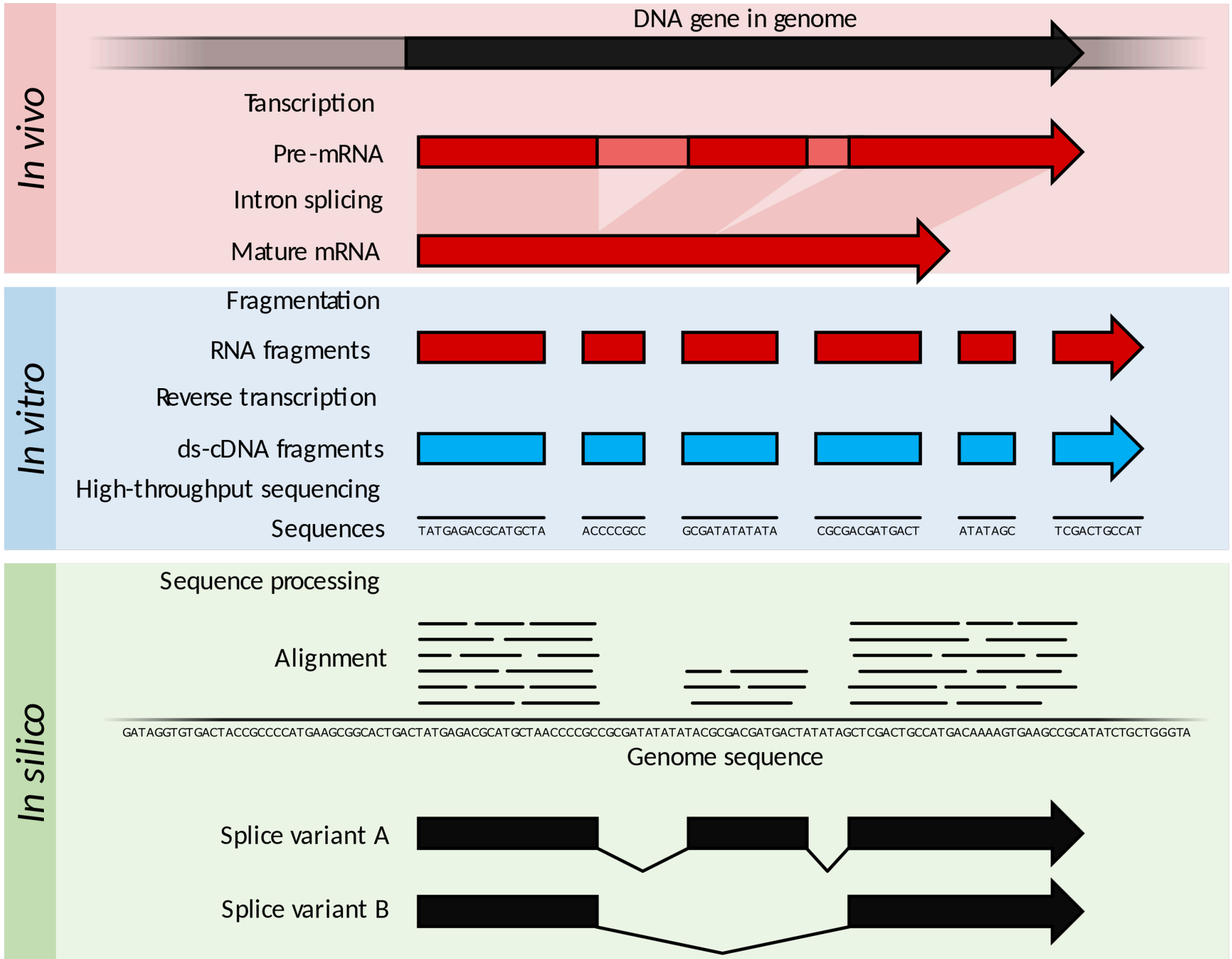


A single read may occur more than once in a reference genome, due to gene/chromosome duplication or repetitive elements

Reads may be assigned to one random location

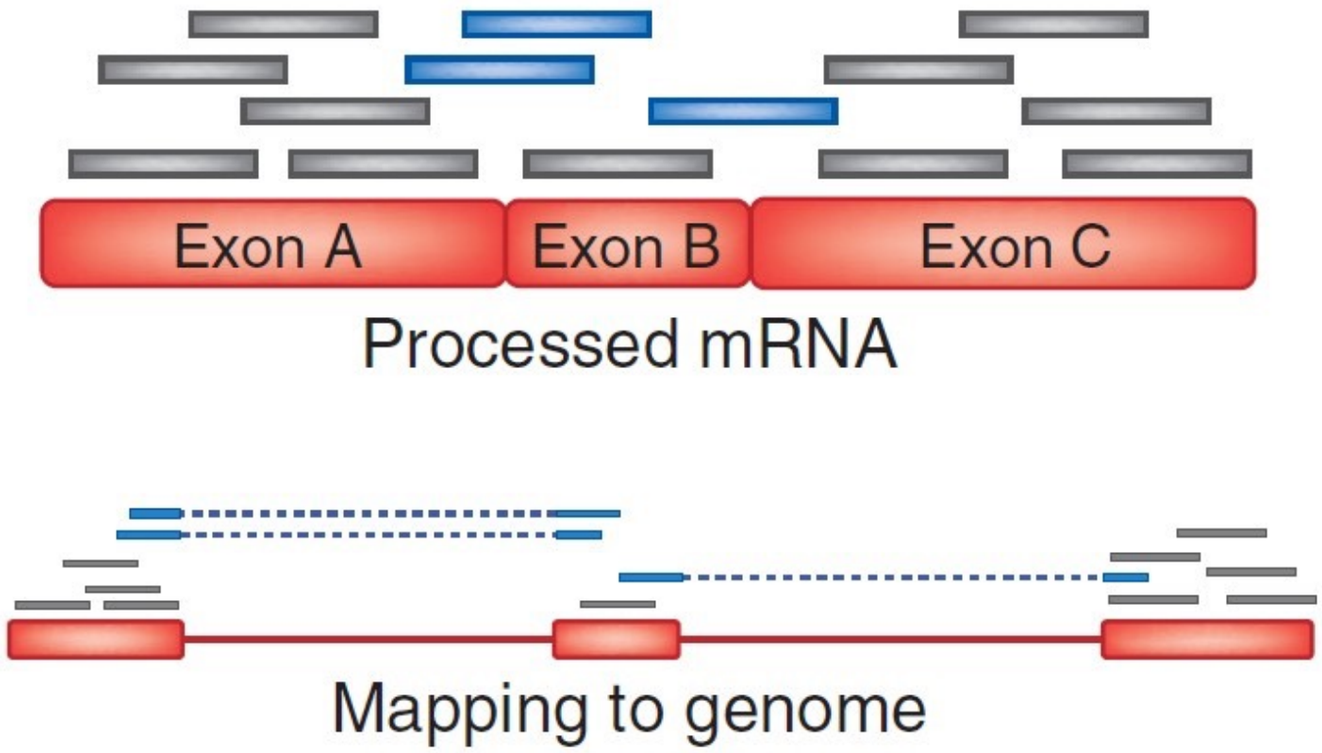
Affects mapping quality

RNAseq and spliced-read mapping



From Wikipedia

RNAseq and spliced-read mapping



There are specialised aligners for RNA seq data that take spliced reads into account

- E.g. TopHat, SubRead, Star

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
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@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

Sort order - in this case coordinate based

```
@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
```

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

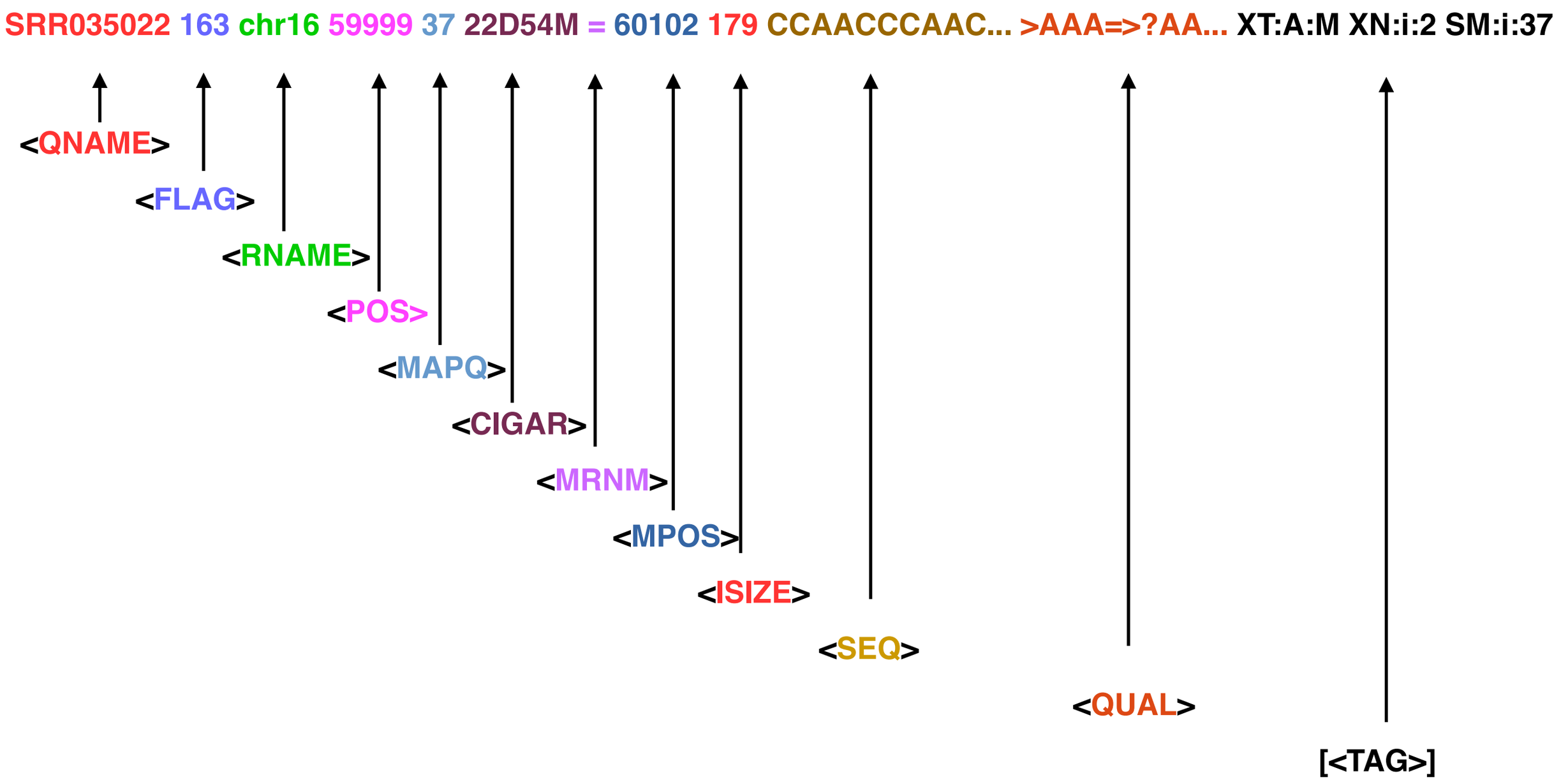
```
@SQ     SN:Ha8  LN:192129815
@SQ     SN:Ha9  LN:253478808
@SQ     SN:Ha10 LN:327788049
@SQ     SN:Ha11 LN:208730832
@SQ     SN:Ha12 LN:208068730
@SQ     SN:Ha13 LN:239367298
@SQ     SN:Ha14 LN:230295834
@SQ     SN:Ha15 LN:202246870
@SQ     SN:Ha16 LN:226777971
@SQ     SN:Ha17 LN:267415242
@SQ     SN:Ha0_73Ns     LN:359367108
```

Read group (@RG) information

```
@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 --
```

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)

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

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