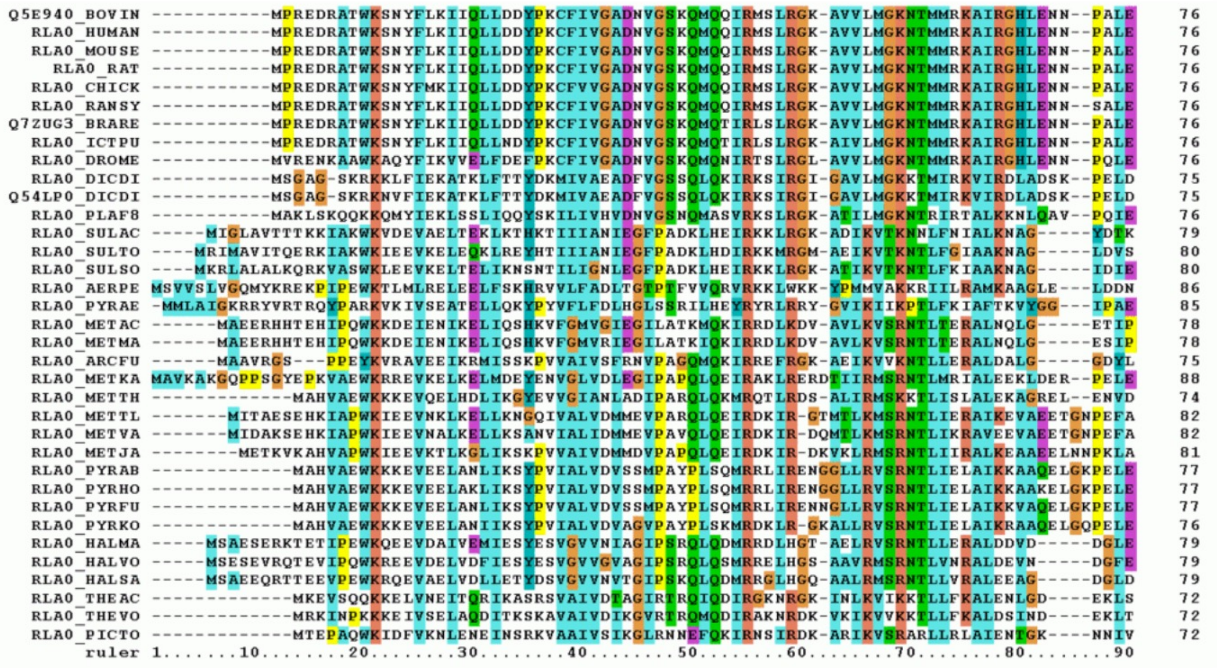
TOPIC 4: Sequence alignment

Learning Outcomes

- 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

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
- Mathematical approach

  -Dynamic programming (slow, but optimal)
- Heuristic methods (fast, but approximate)

  -BLAST, short read aligners

Think-pair-share

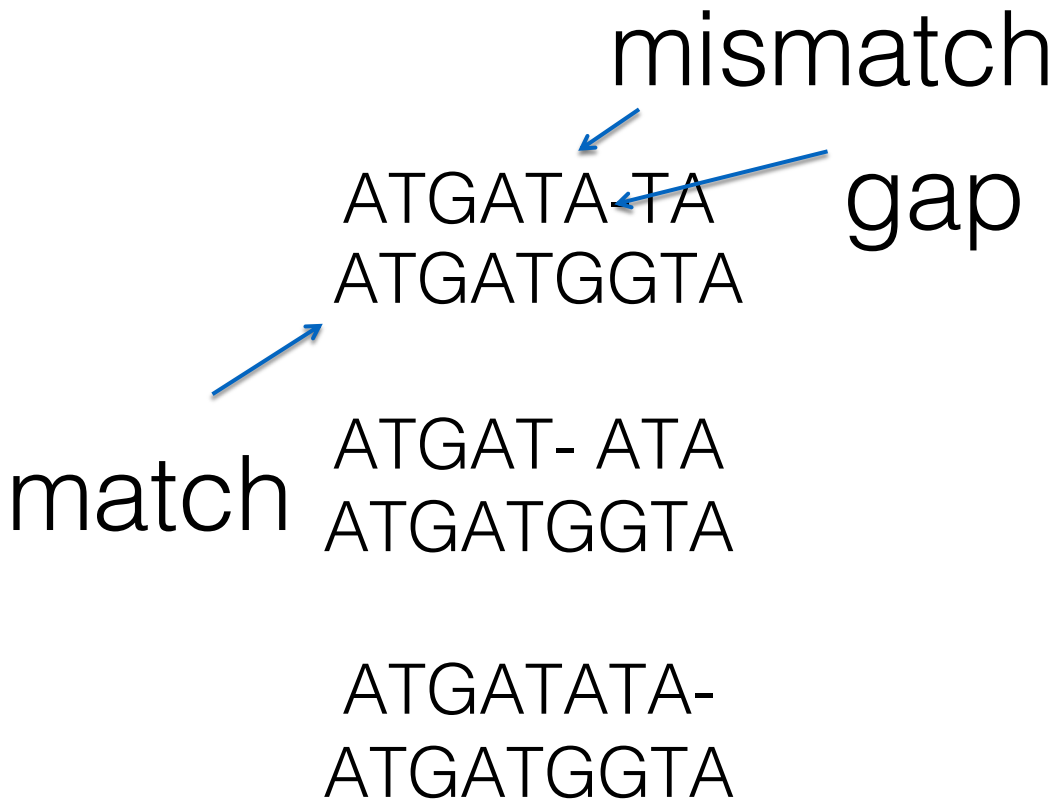
How could you quantify the quality of these DNA alignments?

Example:
ATGATA-TA
ATGATGGTA

ATGAT- ATA
ATGATGGTA

ATGATATA-
ATGATGGTA

scoring matrix: a table of values that describes the probability of a biologically meaningful amino-acid or nucleotide residue-pair occurring in an alignment.

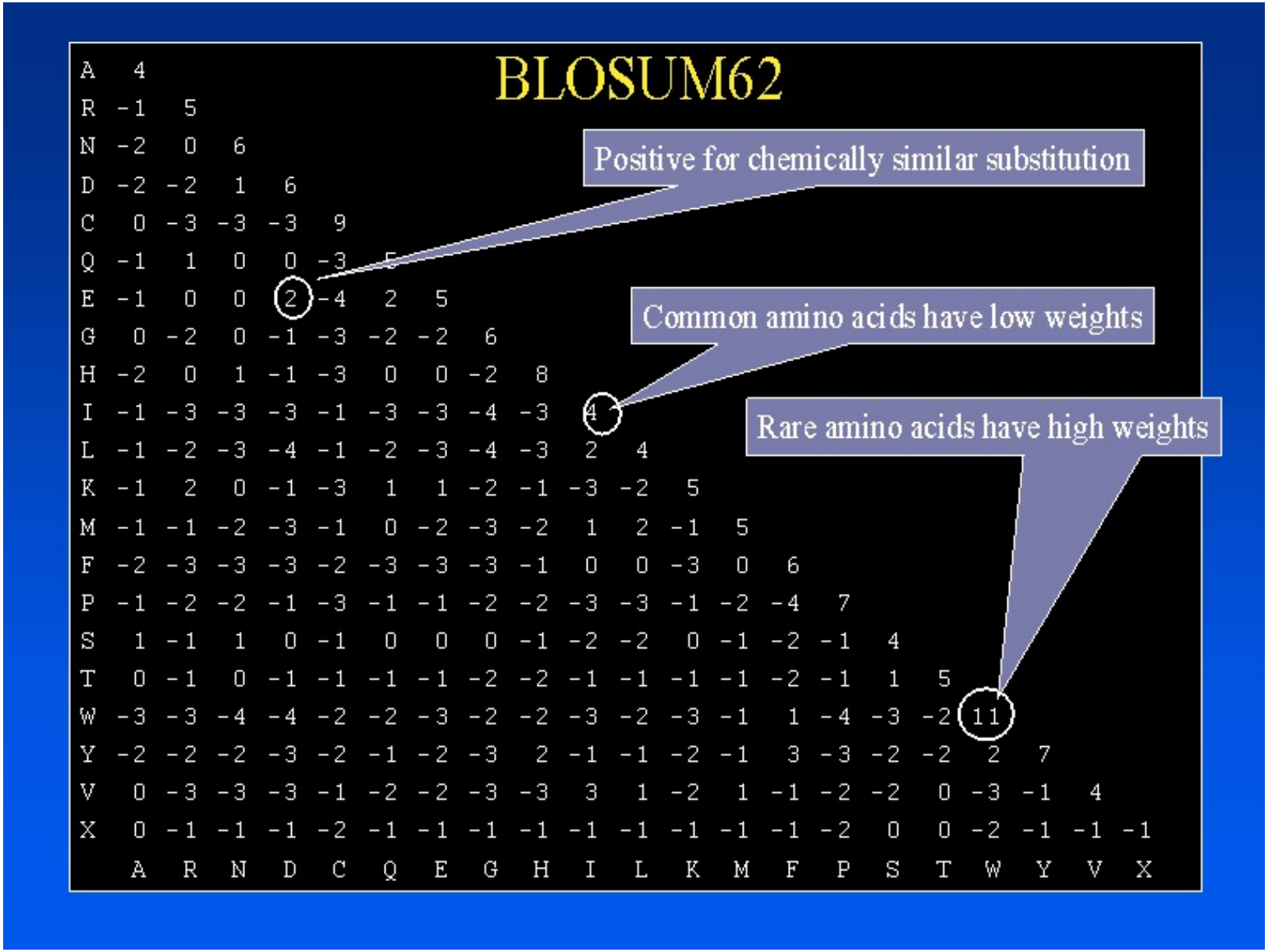


	A	T	G	C
A	1	-1	-1	-1
T	-1	1	-1	-1
G	-1	-1	1	-1
C	-1	-1	-1	1

Gap = -2

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.

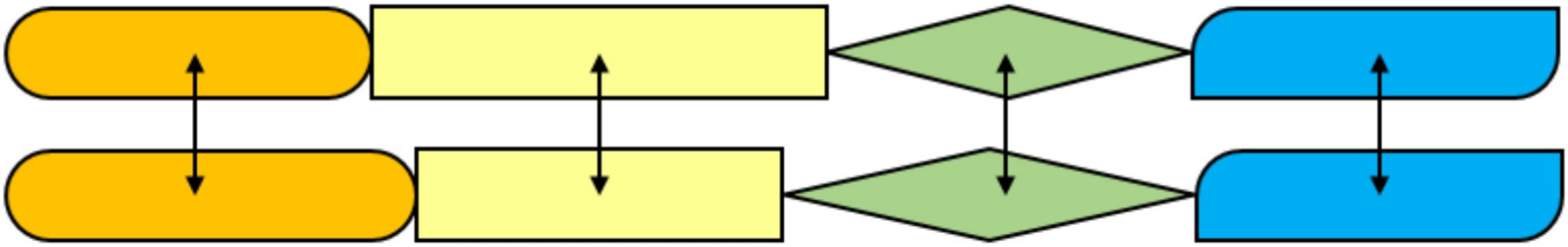

BLOSUM62
Positive for chemically similar substitution
Common amino acids have low weights
Rare amino acids have high weights
A 4
R -1 5
N -2 0 6
D -2 -2 1 6
C 0 -3 -3 -3 9
Q -1 1 0 0 -3 5
E -1 0 0 2 -4 2 5
G 0 -2 0 -1 -3 -2 -2 6
H -2 0 1 -1 -3 0 0 -2 8
I -1 -3 -3 -3 -1 -3 -3 -4 -3 4
L -1 -2 -3 -4 -1 -2 -3 -4 -3 2 4
K -1 2 0 -1 -3 1 1 -2 -1 -3 -2 5
M -1 -1 -2 -3 -1 0 -2 -3 -2 1 2 -1 5
F -2 -3 -3 -3 -2 -3 -3 -3 -1 0 0 -3 0 6
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4 7
S 1 -1 1 0 -1 0 0 0 -1 -2 -2 0 -1 -2 -1 4
T 0 -1 0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1 1 5
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1 1 -4 -3 -2 11
Y -2 -2 -2 -3 -2 -1 -2 -3 2 -1 -1 -2 -1 3 -3 -2 -2 2 7
V 0 -3 -3 -3 -1 -2 -2 -3 -3 3 1 -2 1 -1 -2 -2 0 -3 -1 4
X 0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2 0 0 -2 -1 -1 -1
A R N D C Q E G H I L K M F P S T W Y V X

Gap penalties

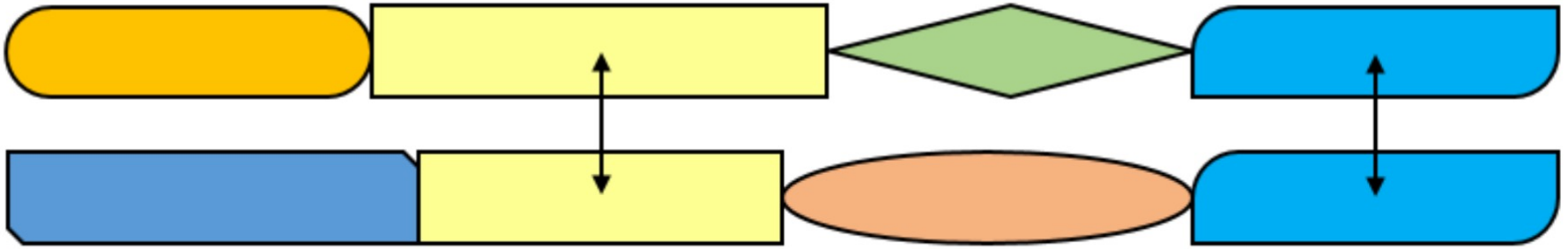
- Too little gap penalty gives nonsense non-homologous alignments.
- Gaps are common, so too high gap penalty removes real alignments.
- “Affine” gap penalty has a large penalty to introduce a gap and a smaller penalty to extend one.

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

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).

- Local alignment algorithms finds the region (or regions) of highest similarity between two sequences and build the alignment outward from there (Smith-Waterman).

- Demo of the two algorithms: https://gtuckerkellogg.github.io/pairwise/demo/

- Video demonstrating exactly how to solve the Needleman-Wunsch alignment by hand https://www.youtube.com/watch?v=ipp-pNRIp4g

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)

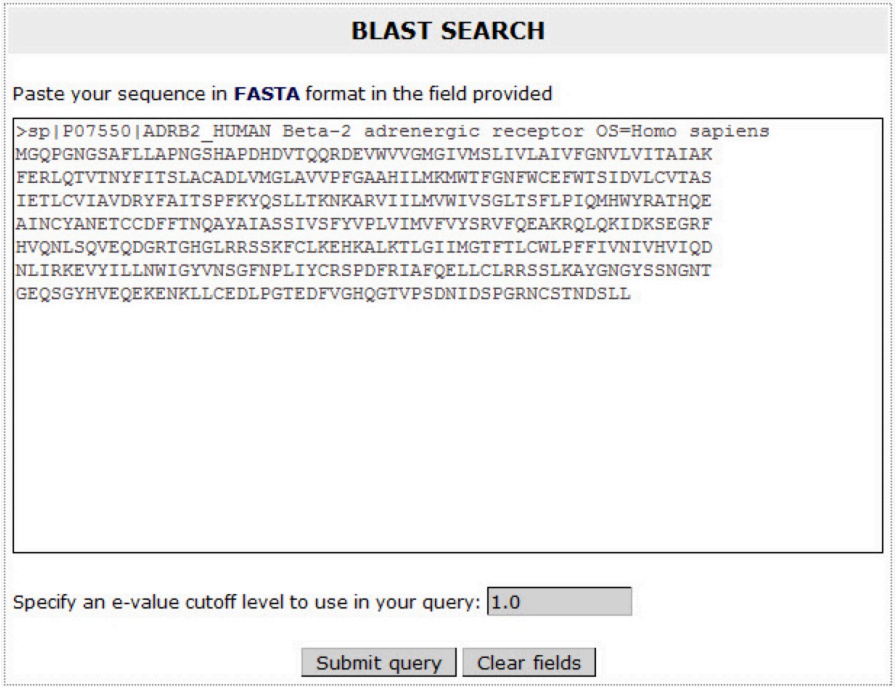
		A	T	G	C	T
	0	-2	-4	-6	-8	-10
A	-2	1	-1	-3	-5	-7
G	-4	-1	0	0	-2	-4
C	-6	-3	-2	-1	1	-1
T	-8	-5	-2	-3	-1	2

https://www.youtube.com/watch?v=ipp-pNRIp4g

Basic Local Alignment Search Tool (BLAST)

Used for finding alignments between a small number of sequences and a large database

query vs database



Examples:

nr = non-redundant

Swiss Prot

Model organisms (TAIR)

Your favorite sequences

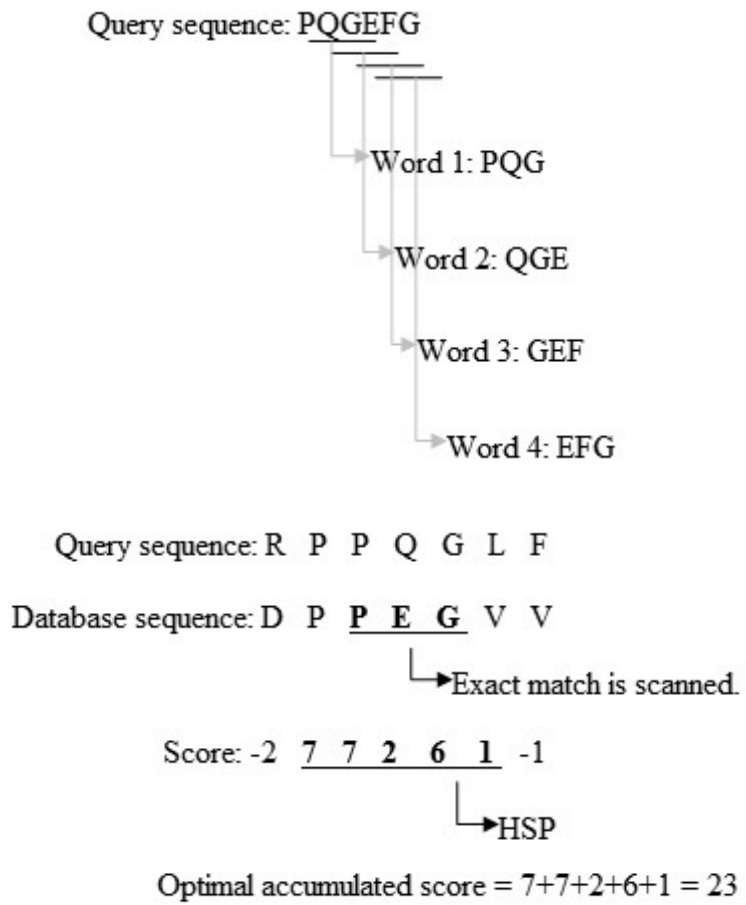
http://www.ncbi.nlm.nih.gov/books/NBK21097/

BLAST

BLAST searches for regions of similarity and doesn't try to align the entire sequence (local alignment). A global alignment aligns two sequences over the entire length

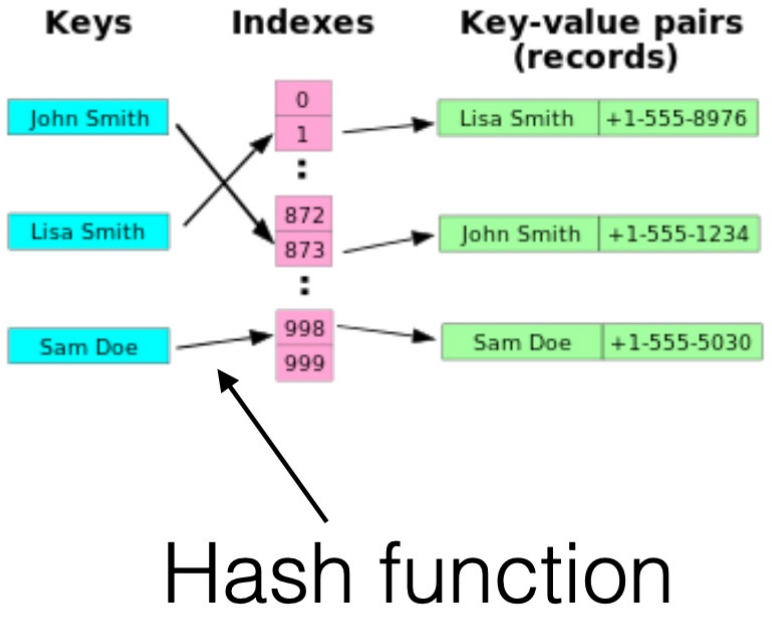
Designed to identify homologous sequences.

Hashed seed-extend algorithm



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



Step 1. Divide up your query sequence into words (typically 11 for DNA and 3 for proteins)

query sequence LIAWHCMPNAAA

list of words

LIA
IAW
AWH
WHC

Step 2. Find sequences in the database that have matches to query words with a score greater than your **threshold**

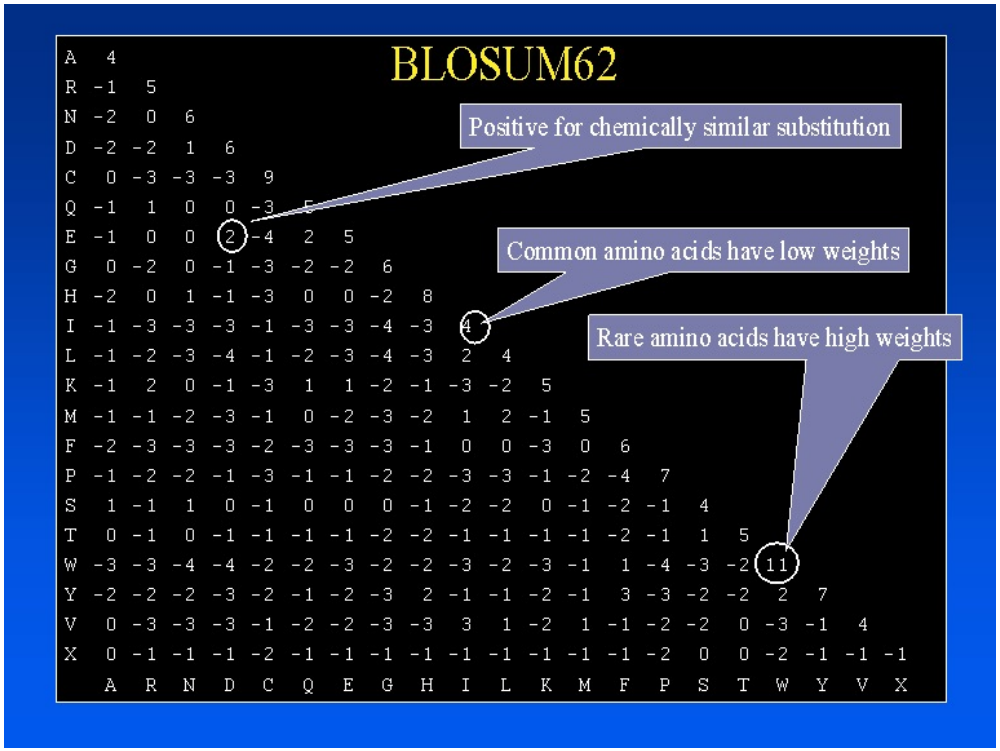
query sequence LIAWHCMPNAAA

query word WHC

database sequence 1 …WHC…

database sequence 2 ….WIC…

database sequence 3 …AIC…



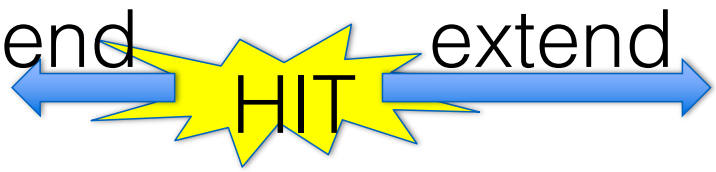
What is the score for each of these alignments (use the BLOSUM62 scoring matrix)?

Step 3. If a match is found extend the alignment around it in each direction for as long as the cumulative score is above a threshold (e.g. 0) or if it falls off by more than X from its maximum. (or you get to the end of the sequence)

This extended match is called a High Scoring Pair (HSP)

query sequence LIAWHCMPNAAA

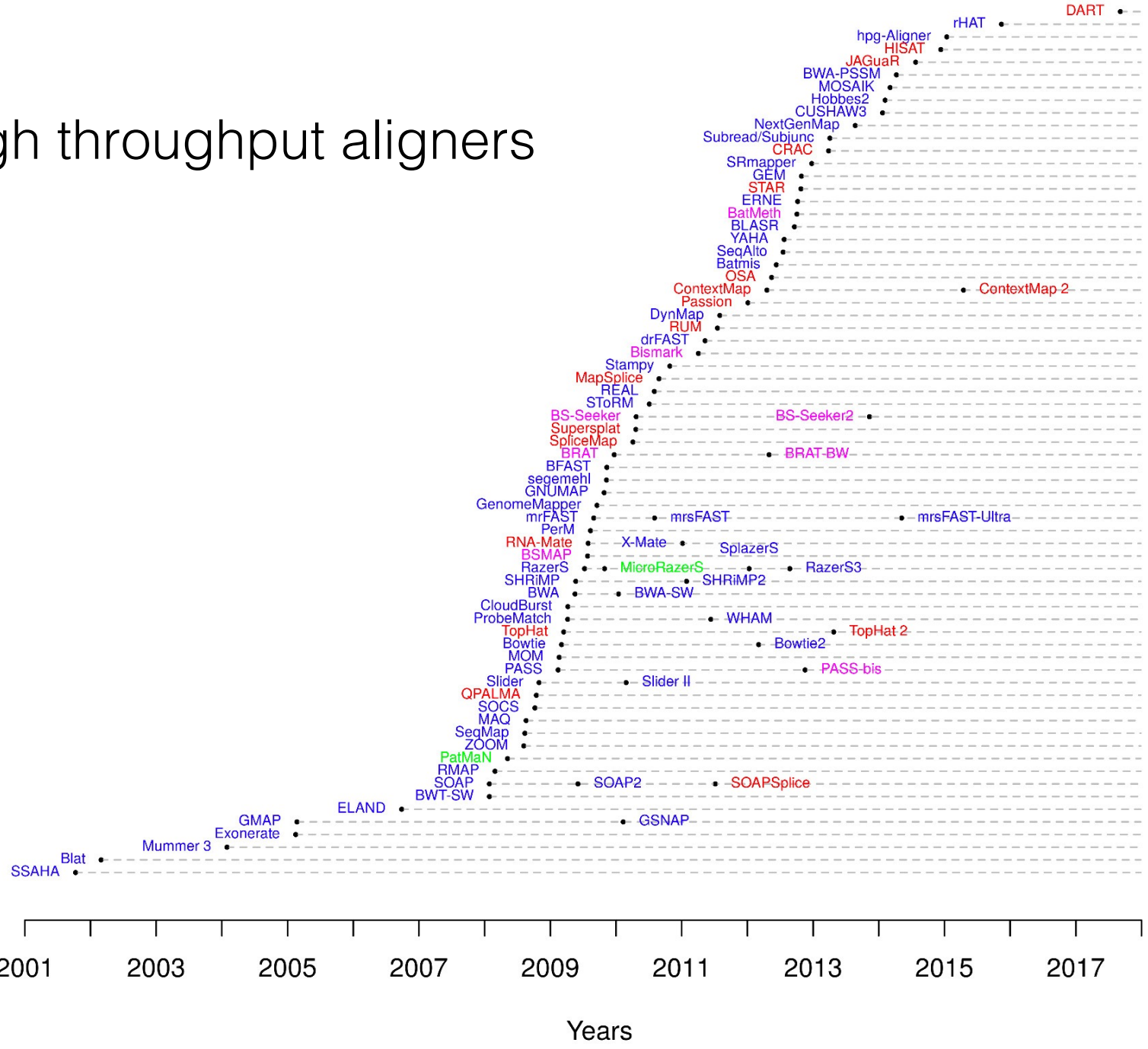
database sequence 1 IIAWHCMPNDDA



BLAST

- Why not use BLAST for short read data (e.g., aligning short reads to a reference)?
  - Typically takes 0.1 to 1 second to search 1 sequence against a database
  - 60 million reads equates to 70 CPU days

High throughput aligners

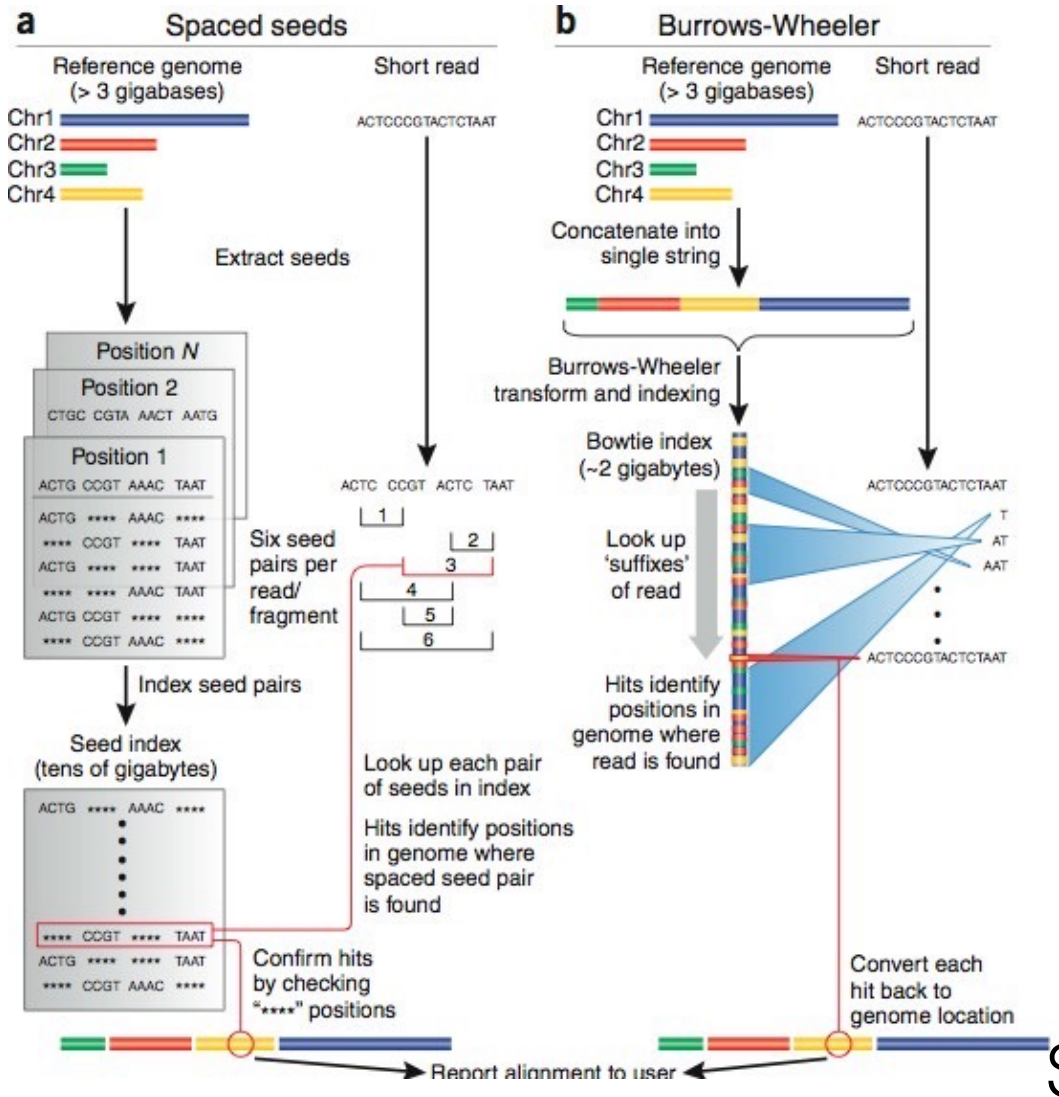


http://www.ebi.ac.uk/~nf/hts_mappers/

Short read alignment is hard

- Billions of short sequences aligned to a very long reference
- Short reads contain less information and are less likely to have a unique mapping location

Approaches to align short reads



Trapnell & Salzberg 2009

Hashed seed-extend algorithms

- 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

Reference sequence:

…GATCTCGATCGATGATCGTAGGATTGATCAGCTA…

Short read:

TCGATCGATGATCGAAGGATTGATCAG

Seed-extend algorithm

Reference sequence:

…GATCTCGATCGATGATCGTAGGATTGATCAGCTA…

Short read:

TCGATCGAT GATCGAAGG ATTGATCAG

9bp seed 9bp seed 9bp seed

The algorithm will try to match each seed to the reference.
If there is a match with any seed, it performs a local alignment

Seed-extend algorithm

Reference sequence:

seed ->Extend with Smith-Waterman->

...GATCTCGATCGATGATCGTAGGATTGATCAGCTA...

TCGATCGATGATCGAAGGATTGATCAG

Short read:

TCGATCGAT GATCGAAGG ATTGATCAG

9bp seed 9bp seed 9bp seed

Here there is a match with at least one seed

Seed-extend algorithm

Reference sequence:

…GATCTCGATCGATGATCGTAGGATTGATCAGCTA…

Short read:

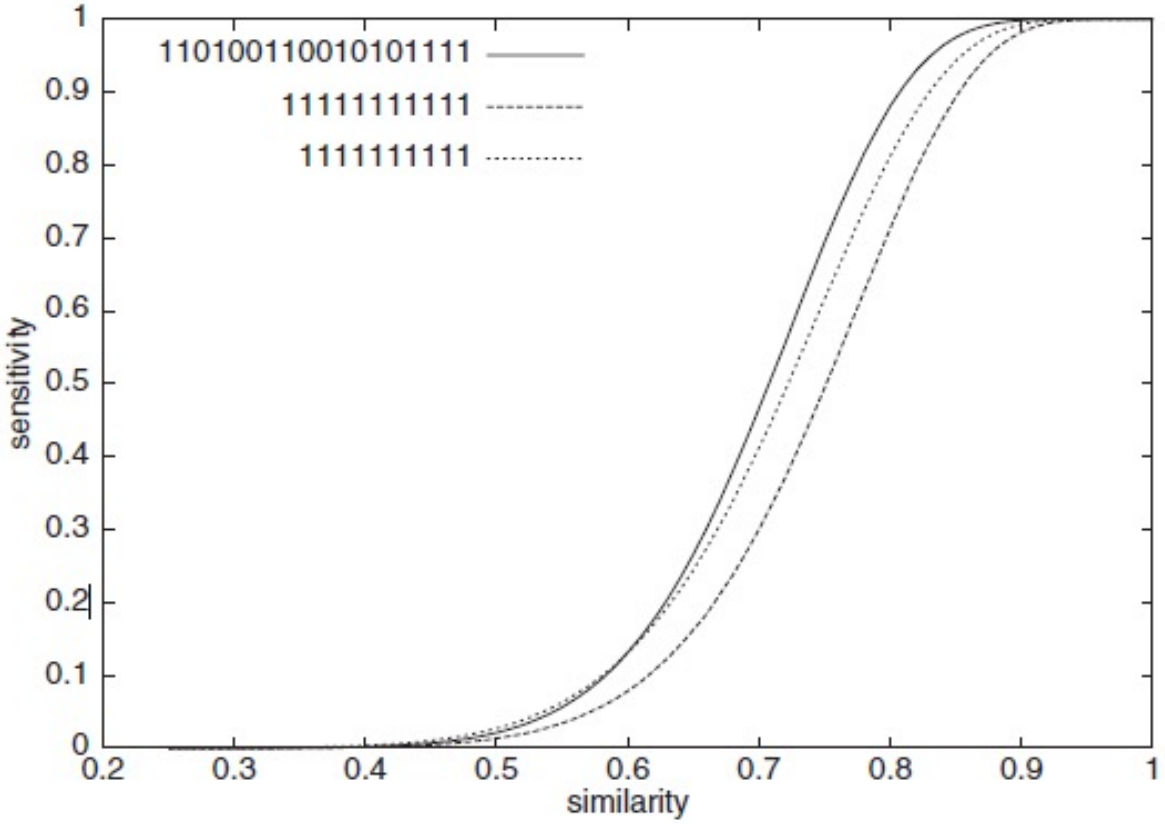
TAGATCGAT GATCGAAGG ATTGAGCAG

9bp seed 9bp seed 9bp seed

With three sequencing errors/SNPs, there can be no matches

Spaced seeds

- To increase sensitivity we can used spaced-seeds:



Spaced seeds

- To increase sensitivity we can used spaced-seeds:

```
111111111
GATAGCTAGCTAAT
  AGCTAGCTA
```

Consecutive seed template with **length** 9bp

Reference

Query

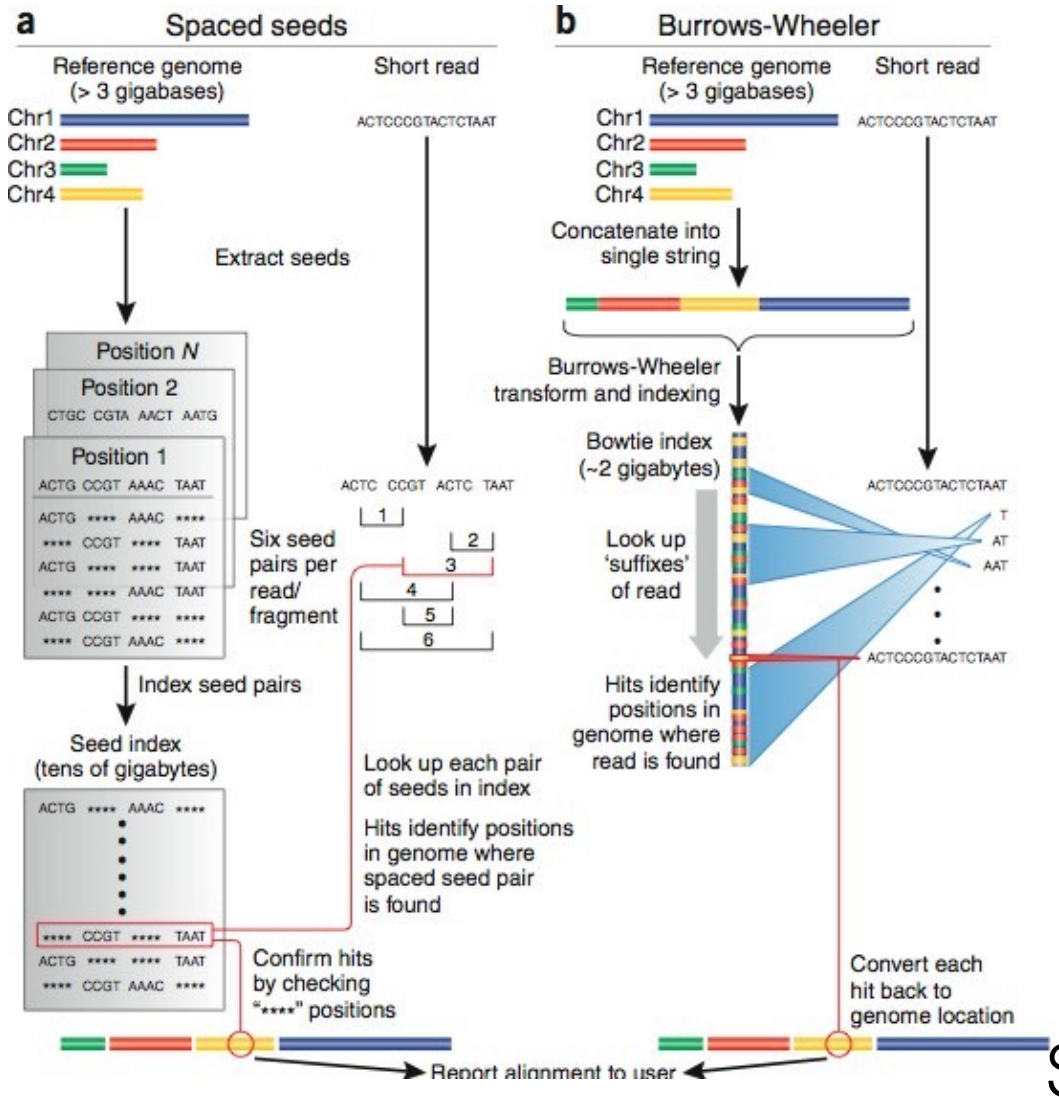
```
101011010111011
GATAGCTAGCTAAT
GATAGCGAGCTAAT
```

Consecutive seed template with **weight** 9bp

Reference

Query

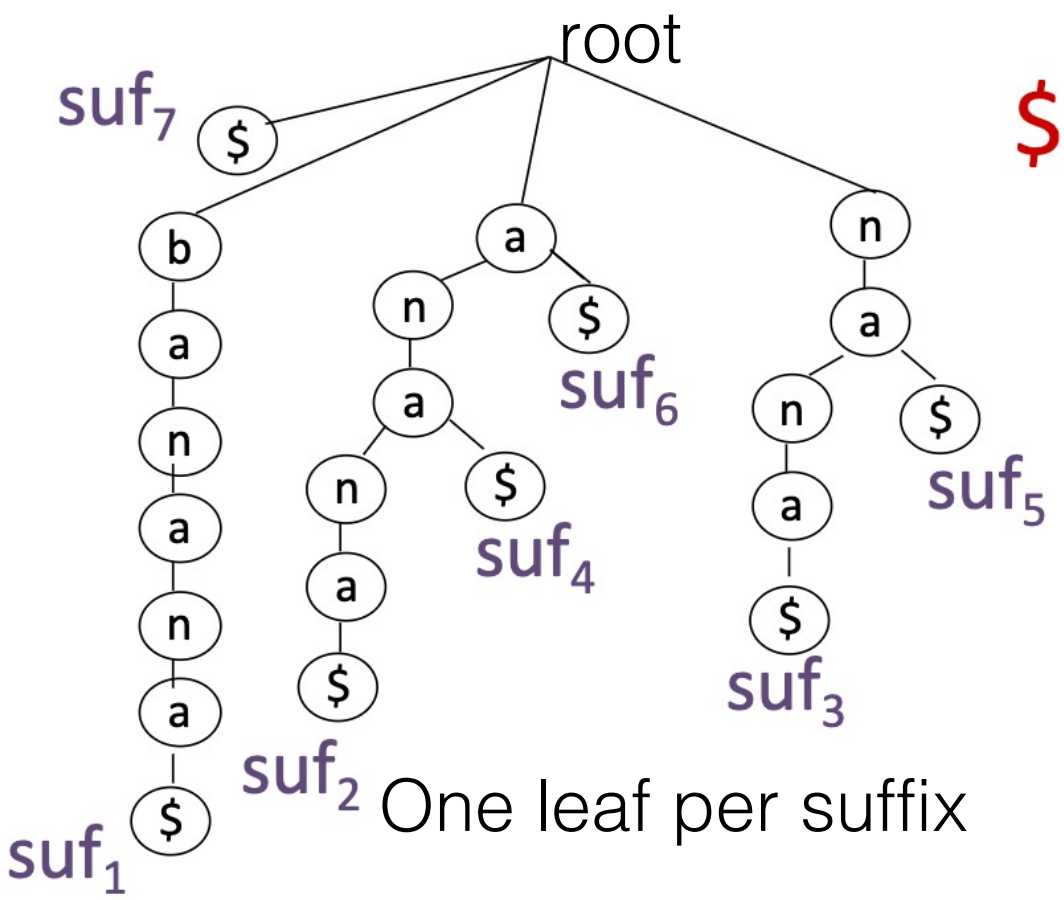
Approaches to align short reads



Trapnell & Salzberg 2009

Suffix-Trie

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



T = banana$

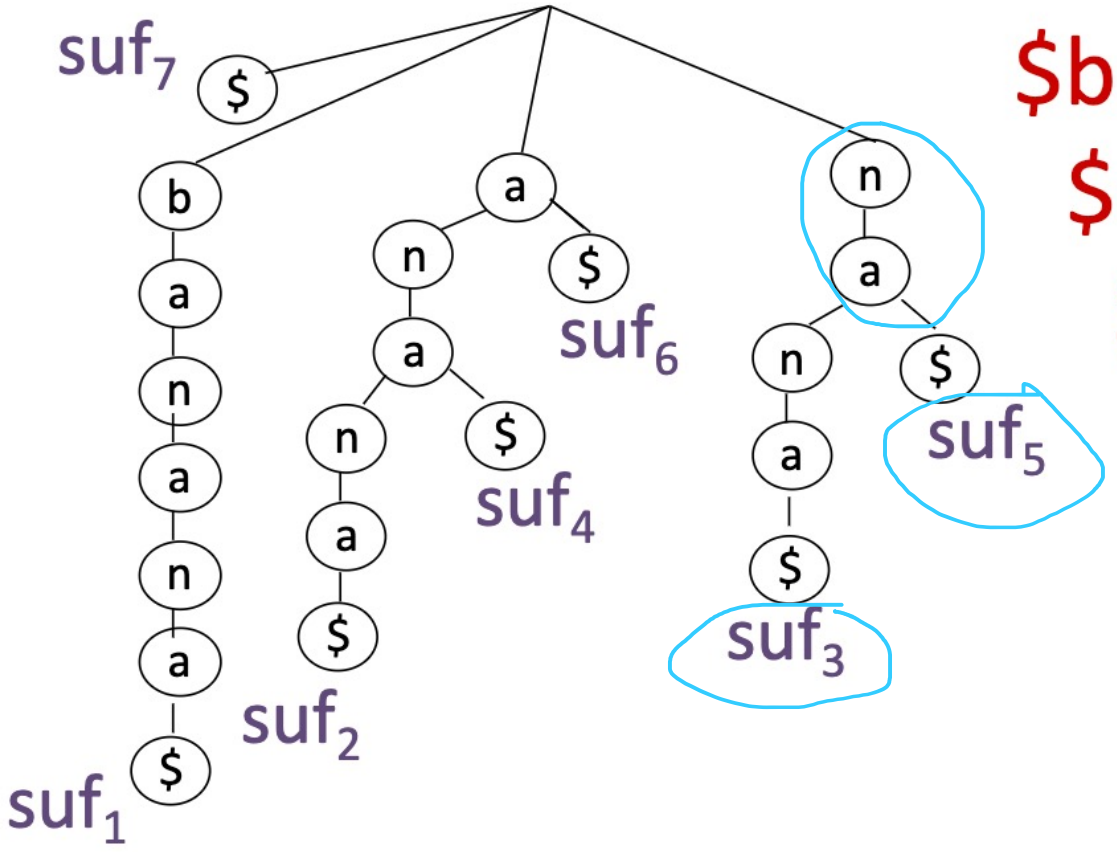
$banana suf1
$anana suf2
$nana suf3
$ana suf4
$na suf5
$a suf6
$ suf7

Combining edge labels on the path from the root to the leaf *i* spells out the suffix that begins at position *i*

Suffix-Trie

Search “na”

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



T = banana$

$banana suf1
$anana suf2
$nana suf3
$ana suf4
$na suf5
$a suf6
$ suf7

Suffix tries allow particularly fast implementations of many important string operations (eg. search for substrings quickly)

Try out this website 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 storage/memory requirements (e.g. Human genome can be stored in 2Gb of RAM)
  - Burrows Wheeler Transform to perform fast lookups

More information about the BWT:
https://www.youtube.com/watch?v=4n7NPk5lwbI

Burrows-Wheeler Transform (BWT)

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

i	Suffix	Sorted Suffix	SA[i]	ISA[i]	Sorted Rotations	BWT[i]
0	banana$	$	6	4	**$**banana	a
1	anana$	a$	5	3	**a$**banan	n
2	nana$	ana$	3	6	**ana$**ban	n
3	ana$	anana$	1	2	**anana$**b	b
4	na$	banana$	0	5	**banana$**	$
5	a$	na$	4	1	**na$**bana	a
6	$	nana$	2	0	**nana$**ba	a

Burrows-Wheeler Transform (BWT)

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

i	Suffix	Sorted Suffix	SA[i]	ISA[i]	Sorted Rotations	BWT[i]
0	banana$	$	6	4	**$banan**a	a
1	anana$	a$	5	3	**a$banan**	n
2	nana$	ana$	3	6	**ana$ban**	n
3	ana$	anana$	1	2	**anana$b**	b
4	na$	banana$	0	5	**banana$**	$
5	a$	na$	4	1	**na$bana**	a
6	$	nana$	2	0	**nana$ba**	a

Burrows-Wheeler Transform (BWT)

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

i	Suffix	Sorted Suffix	SA[i]	ISA[i]	Sorted Rotations	BWT[i]
0	banana$	$	6	4	**$banana**$_1$	a
1	anana$	$_1$a$	5	3	**a$banan**$_1$	n
2	nana$	$_2$ana$	3	6	**ana$ban**$_2$	n
3	ana$	$_3$anana$	1	2	**anana$b**$_1$	b
4	na$	$_1$banana$	0	5	**banana$**	$
5	a$	$_1$na$	4	1	**na$bana**$_2$	a
6	$	$_2$nana$	2	0	**nana$ba**$_3$	a

Burrows-Wheeler Transform (BWT)

- Encodes data so that it is easier to compress
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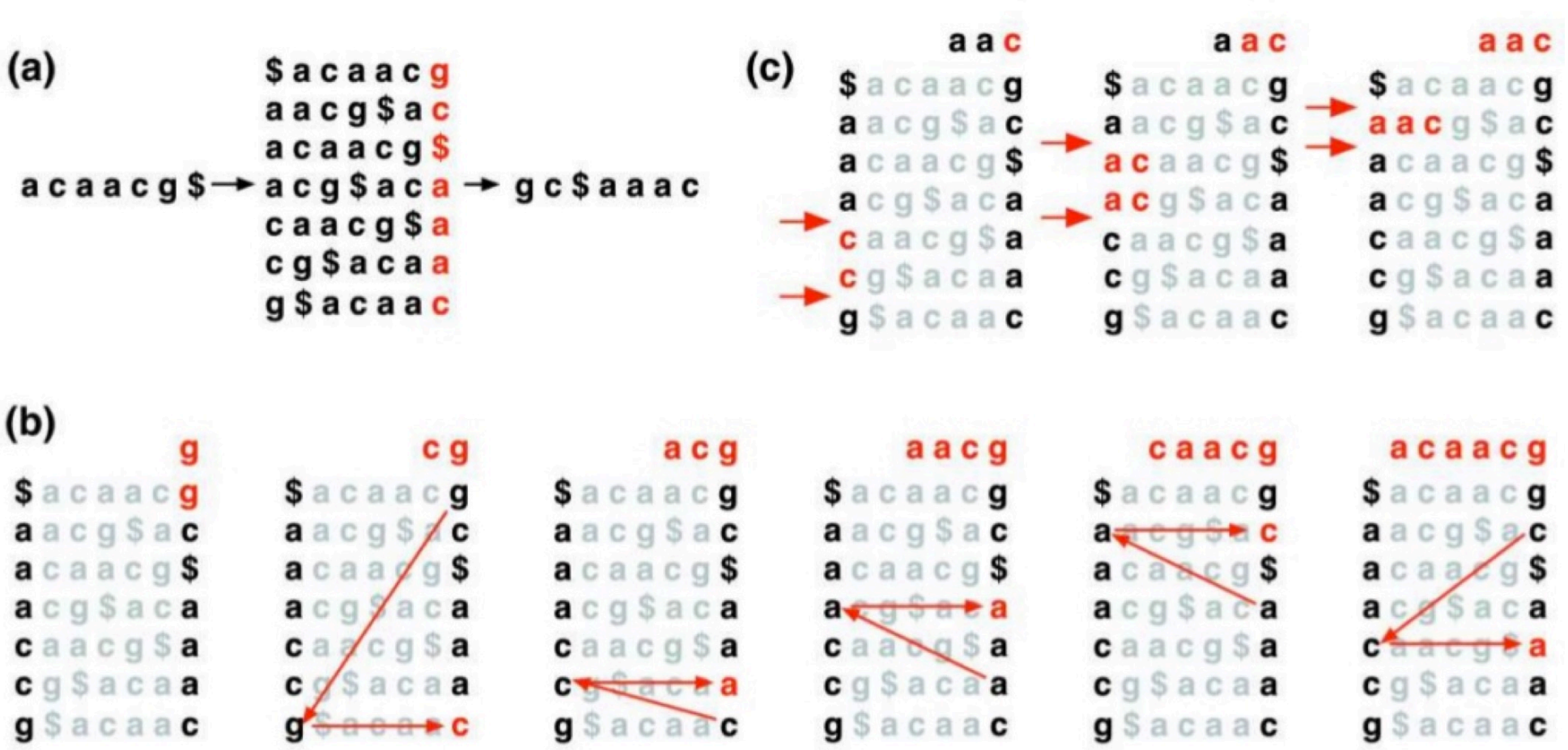
i	Suffix	Sorted Suffix	SA[i]	ISA[i]	Sorted Rotations	BWT[i]
0	banana$	$	6	4	$banana$_1$	a
1	anana$	$_1$a$	5	3	a$banan$_1$	n
2	nana$	$_2$ana$	3	6	anaban_2$	n
3	ana$	$_3$anana$	1	2	anana$b$_1$	b
4	na$	$_1$banana$	0	5	banana$	$
5	a$	$_1$na$	4	1	na$bana$_2$	a
6	$	$_2$nana$	2	0	nanaba_3$	a

Burrows-Wheeler Transform (BWT)

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

i	Suffix	Sorted Suffix	SA[i]	ISA[i]	Sorted Rotations	BWT[i]
0	banana$	$	6	4	$banana$_1$	a
1	anana$	$_1$a$	5	3	a$banan$_1$	n
2	nana$	$_2$ana$	3	6	anaban_2$	n
3	ana$	$_3$anana$	1	2	anana$b$_1$	b
4	na$	$_1$banana$	0	5	banana$	$
5	a$	$_1$na$	4	1	na$bana$_2$	a
6	$	$_2$nana$	2	0	nanaba_3$	a

Burrows-Wheeler Transform



Suffix-Prefix Trie

- Less sensitive for sequences that are more divergent from the reference.
  - Sequencing errors
  - Query - Reference differences

Comparison

Hash referenced spaced seeds (NextGenMap)

- Requires more RAM
- Runs slower
- Simpler to program
- More sensitive

Suffix/Prefix Trie (BWA)

- Requires less RAM
- Runs much faster
- Complicated to program
- Less sensitive

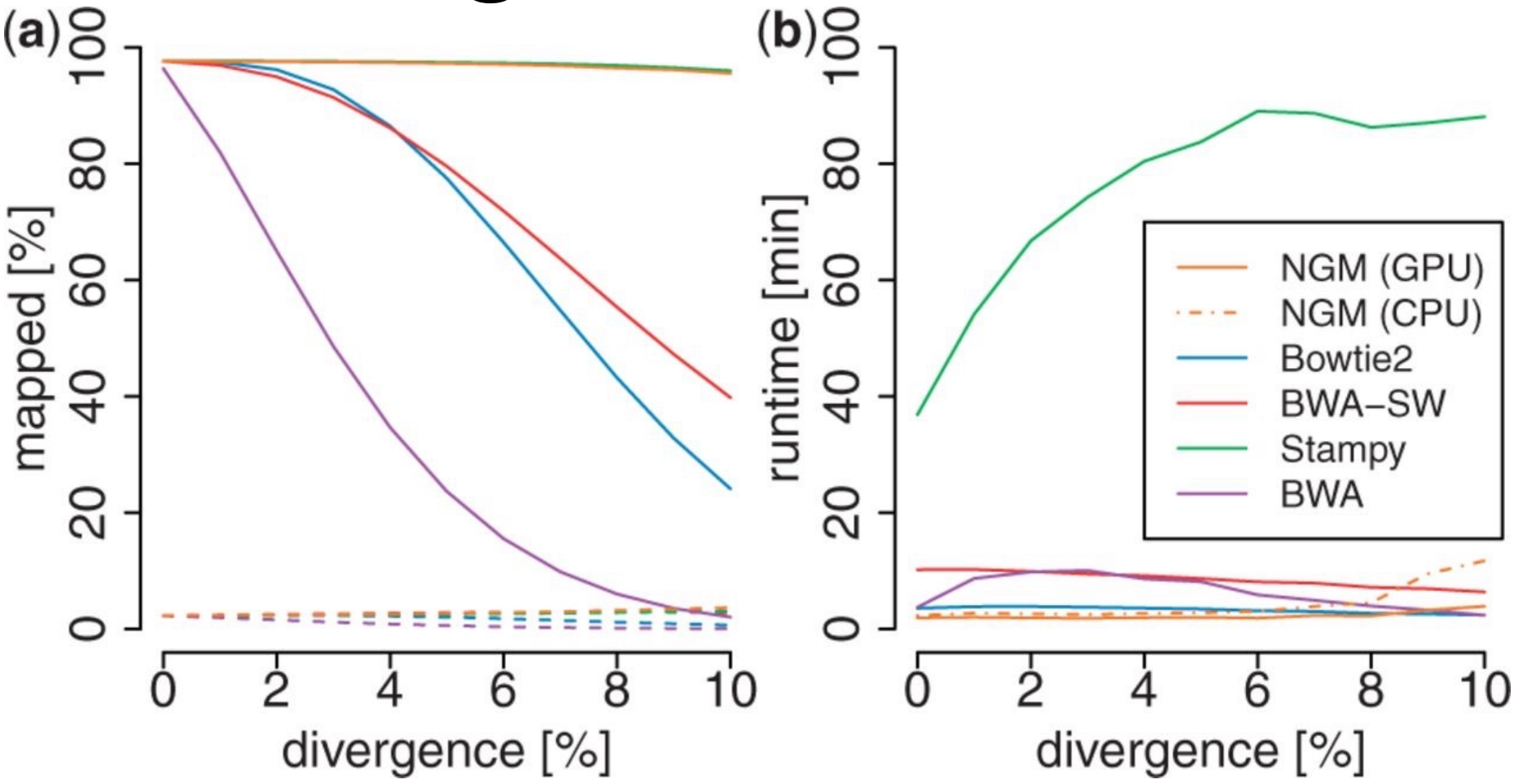
Popular short read aligners

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

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?

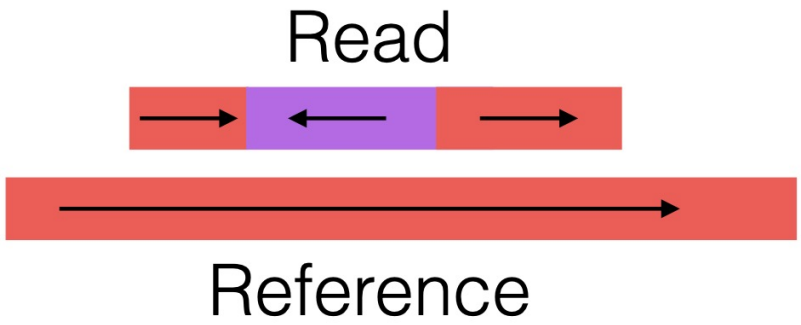
Alignment stats



*From NextGenMap paper

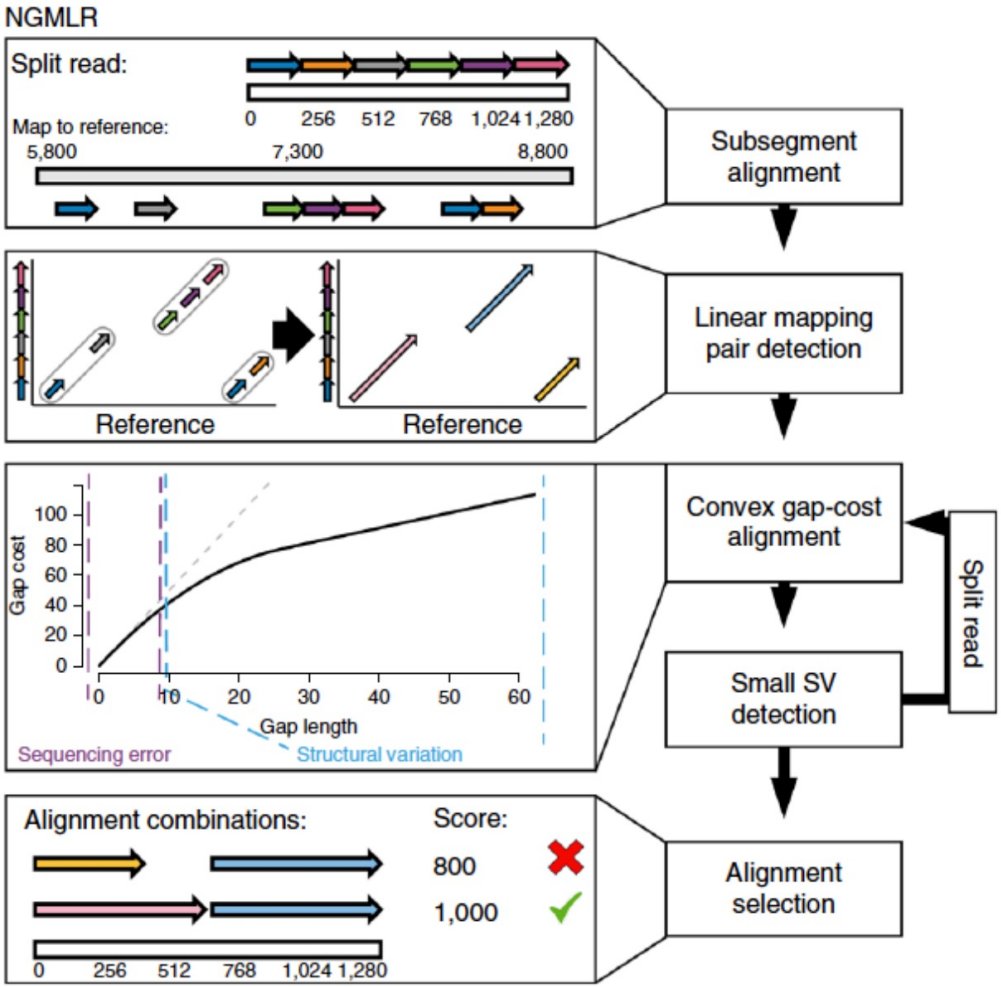
Long read alignment

- 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
- Most long read technologies have higher error rates



Long read alignment

1. Find exact matches between read fragment and reference
2. Look for chains of matches
3. Use local alignment of read to best reference region.



Long read alignment

- Longer reads have more information, but more error.
- Example: **NGMLR** uses k-mers (short strings of a specific length) to pick region and smith-waterman for exact placement.
- 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

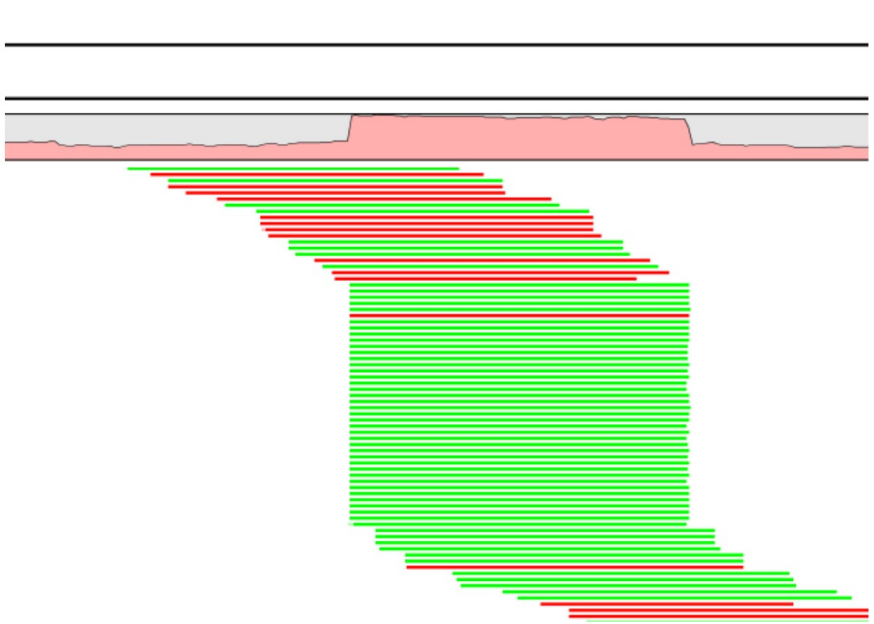
PCR duplicates

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

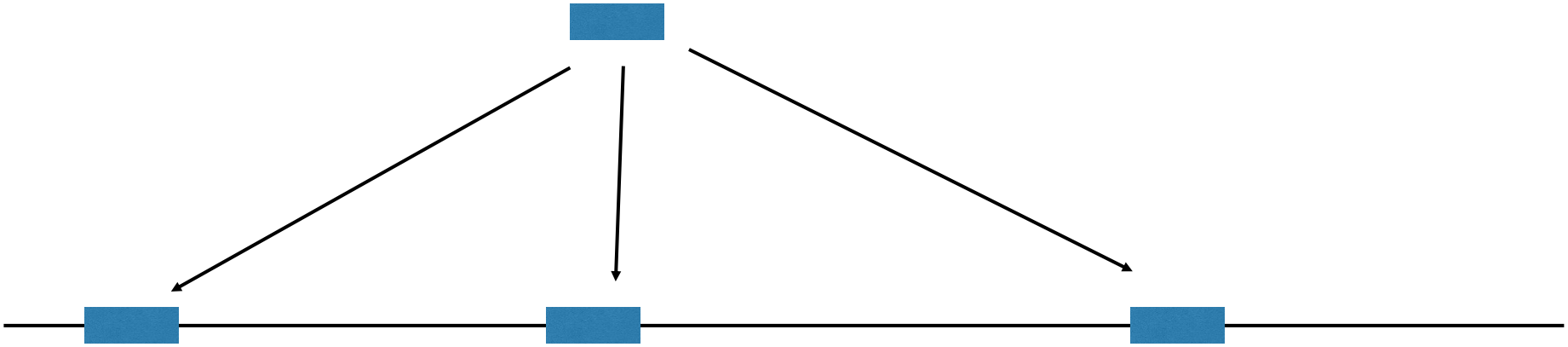
SAMtools and Picard can flag or remove these duplicates based on alignment location

- Samples with same start and stop position are considered duplicates
- Don't flag duplicates for GBS (set start and stop)



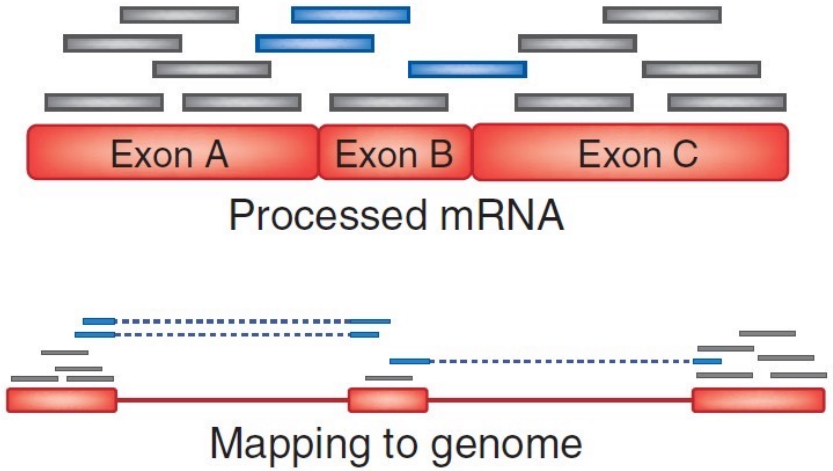
From Qiagen website

Multiple 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

Spliced-read mapping



- Need to account for splicing
- Examples: 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

Keeps a track of the reference genome

And individual read records

SAM format

Header (lines begin with @)

```
@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 --
```

Sort order

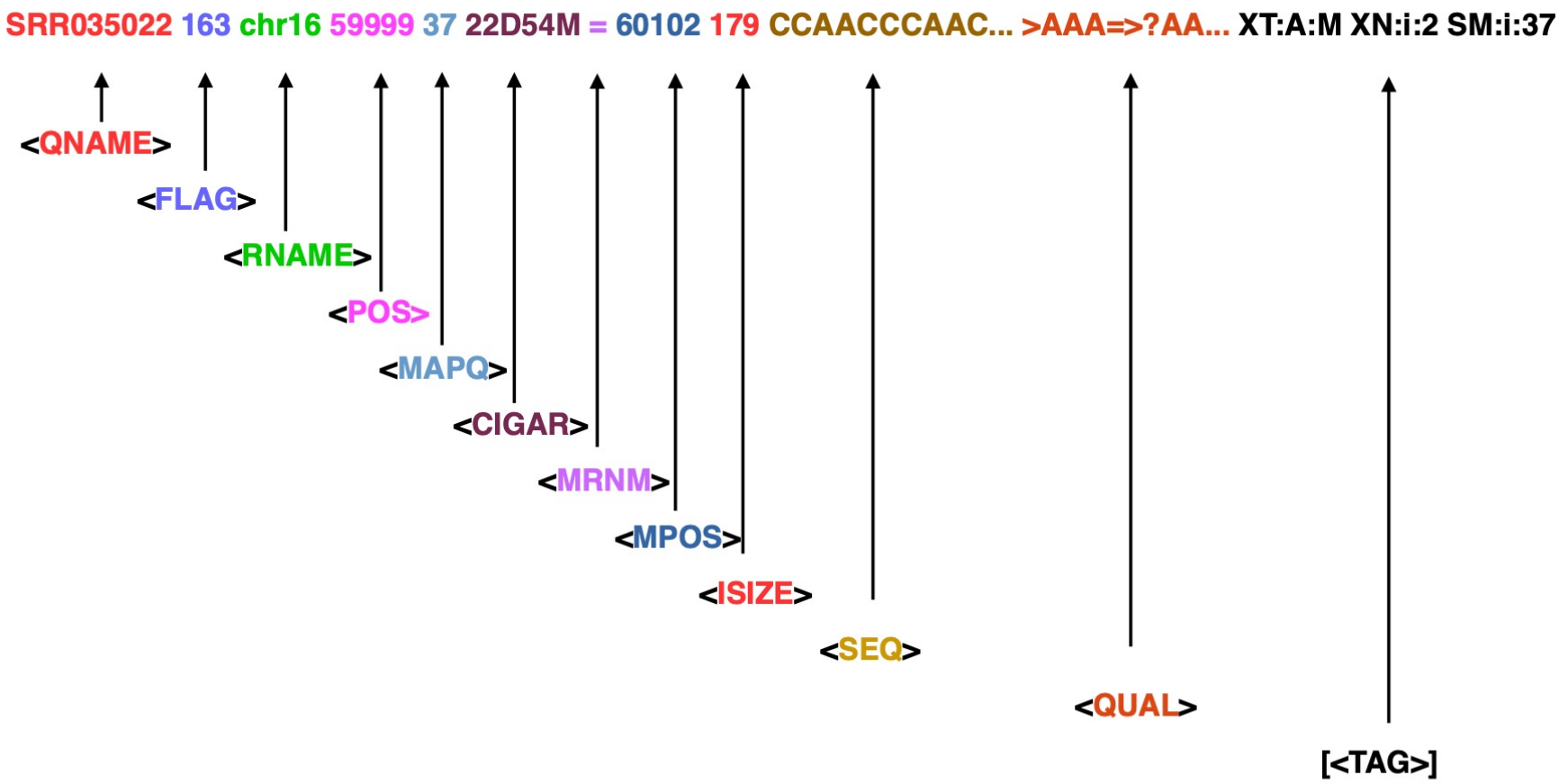
Reference sequence name (SN) and length (LN) e.g. Chromosome Ha7, which is 109,221,022bp long

Read group information (@RG)

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

SAM format

Alignment lines



SAM format

Alignment 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

Background reading

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

- 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