

TOPIC 4: Sequence alignment

Bill 525D - Bioinformatics for Evolutionary Biology

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.

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

Align by hand

TGCAGTT
TGGAATCGTT

In groups, align these two sequences
and justify your result

Alignment by hand

TGCAGTT
TGGAATCGTT

Scoring:

Matching letter: +1

Mismatch letter: -1

Inserting gap: -2

Alignment by hand

TGCA - - - GTT
TGGAATCGTT

Matches

+1+1 +1 +1+1+1

Mismatches

-1

Gaps

-2-2-2

Total score = -1

Scoring:

Matching letter: +1

Mismatch letter: -1

Inserting gap: -2

Alignment by hand

	TGCA--GTT
	TGGAATCGTT
Matches	+1+1 +1 +1+1+1
Mismatches	-1
Gaps	-2-2-2

Total score = -1

Scoring:

Matching letter: +1

Mismatch letter: -1

Inserting gap: -2

What is the best alignment
if the gap penalty is zero?

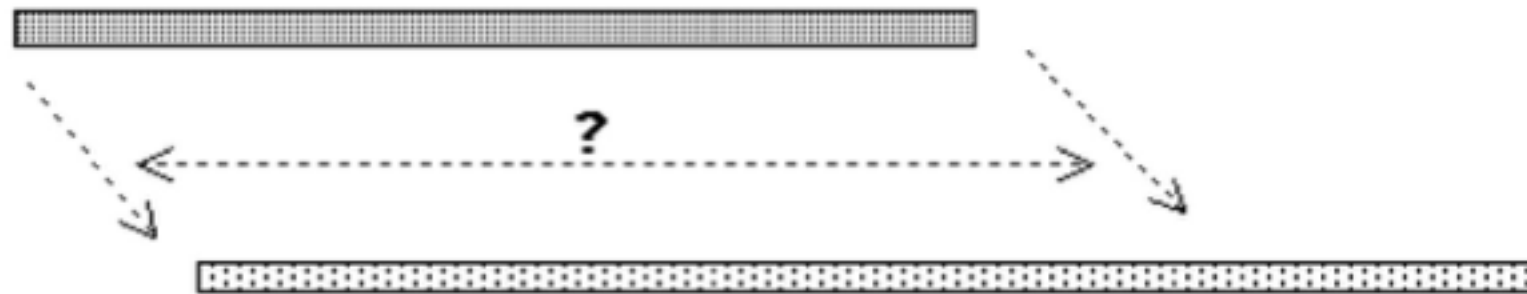
Dynamic programming

- 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

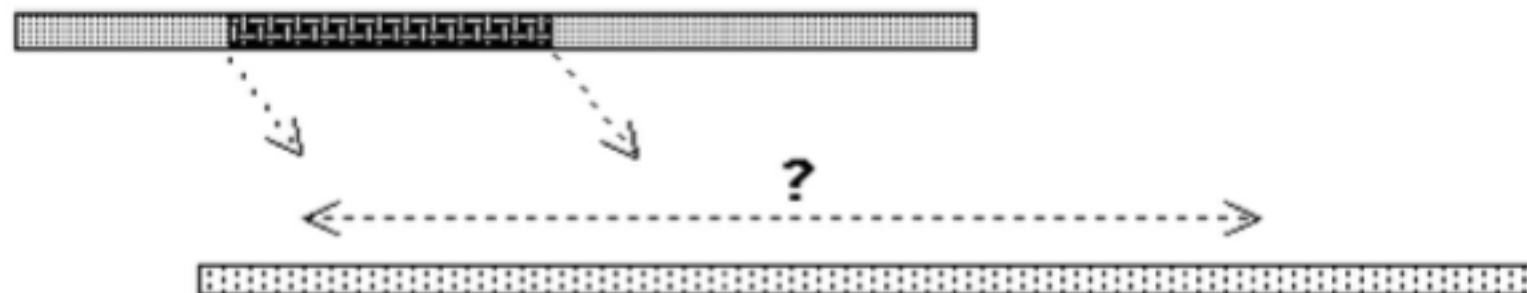
Global vs Local alignments

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

Global Alignment



Local Alignment



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)

Build an alignment path matrix

- For sequences $x(1:i)$ and $y(1:j)$:
 - If $F(i-1, j-1)$, $F(i-1, j)$ and $F(i, j-1)$ are known we can calculate $F(i, j)$
 - Three possibilities:
 - x_i and y_j are aligned, $F(i, j) = F(i-1, j-1) + s(x_i, y_j)$
 - x_i is aligned to a gap, $F(i, j) = F(i-1, j) - d$
 - y_j is aligned to a gap, $F(i, j) = F(i, j-1) - d$
 - The best score up to (i, j) will be the **largest** of the three options
 - d = gap penalty

Dynamic Programming

- Global alignment (Needleman-Wunsch) algorithm
- Example: align GATC to GAC

Scoring system:

Match = +1

Mismatch = -1

Gap = -2

	-	G	A	T	C
-	0				
G					
A					
C					

Dynamic Programming

- Global alignment (Needleman-Wunsch) algorithm
- Example: align GATC to GAC

Scoring system:

Match = +1

Mismatch = -1

Gap = -2

	-	G	A	T	C
-	0	-2	-4	-6	-8
G	-2				
A	-4				
C	-6				

Dynamic Programming

- Global alignment (Needleman-Wunsch) algorithm
- Example: align GATC to GAC

Scoring system:

Match = +1

Mismatch = -1

Gap = -2

	-	G	A	T	C
-	0	-2	-4	-6	-8
G	-2	1			
A	-4				
C	-6				

Dynamic Programming

- Global alignment (Needleman-Wunsch) algorithm
- Example: align GATC to GAC

Scoring system:

Match = +1

Mismatch = -1

Gap = -2

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

Dynamic Programming

- Global alignment (Needleman-Wunsch) algorithm
- Example: align GATC to GAC

Scoring system:

Match = +1

Mismatch = -1

Gap = -2

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

GATC

GA-C

Smith-Waterman local alignment

- Variation on the Needleman-Wunsch algorithm that guarantees best local alignment of any possible length.

Scoring methods

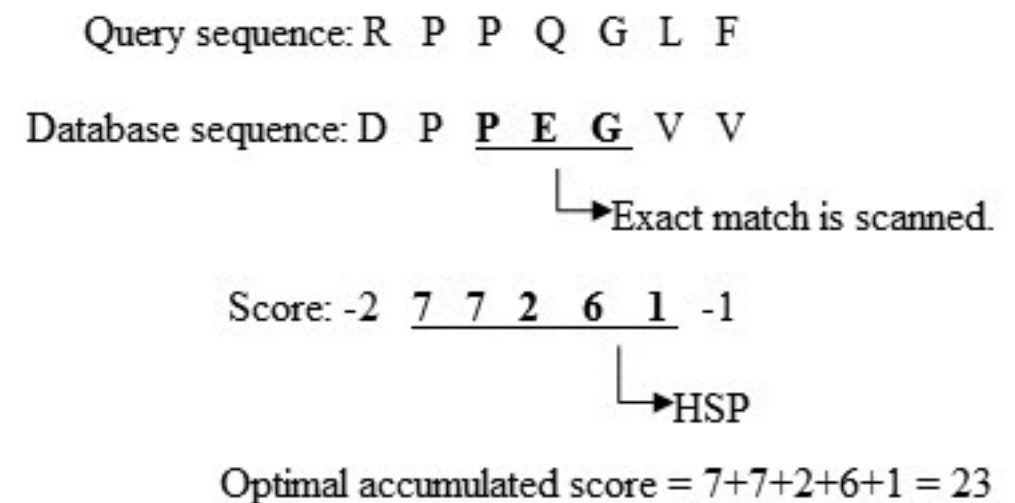
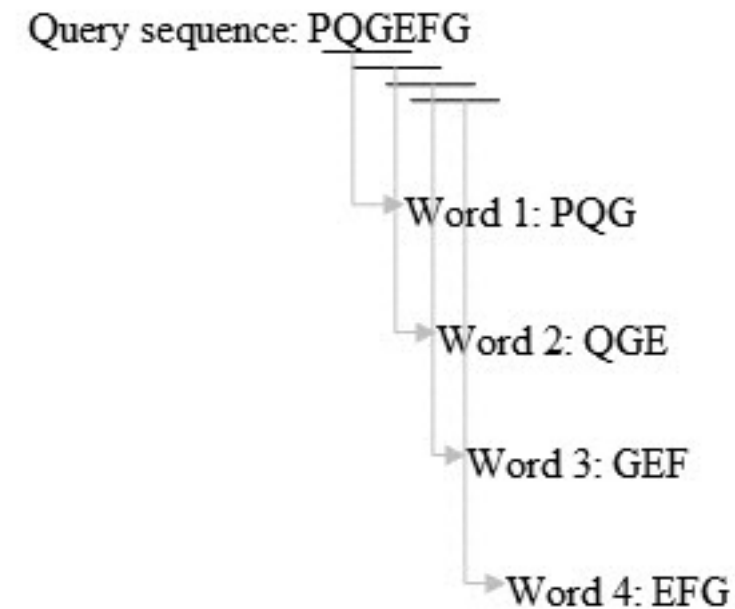
- Scoring systems:
 - Each symbol pairing is assigned a numerical value, based on a symbol comparison table.
 - nucleotides
 - amino acids (PAM, BLOSUM)
- Gap penalties:
 - Opening: The cost of introducing a gap.
 - Extension: The cost to elongate a gap.

Gap penalties

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

BLAST - Best Local Alignment Search Tool

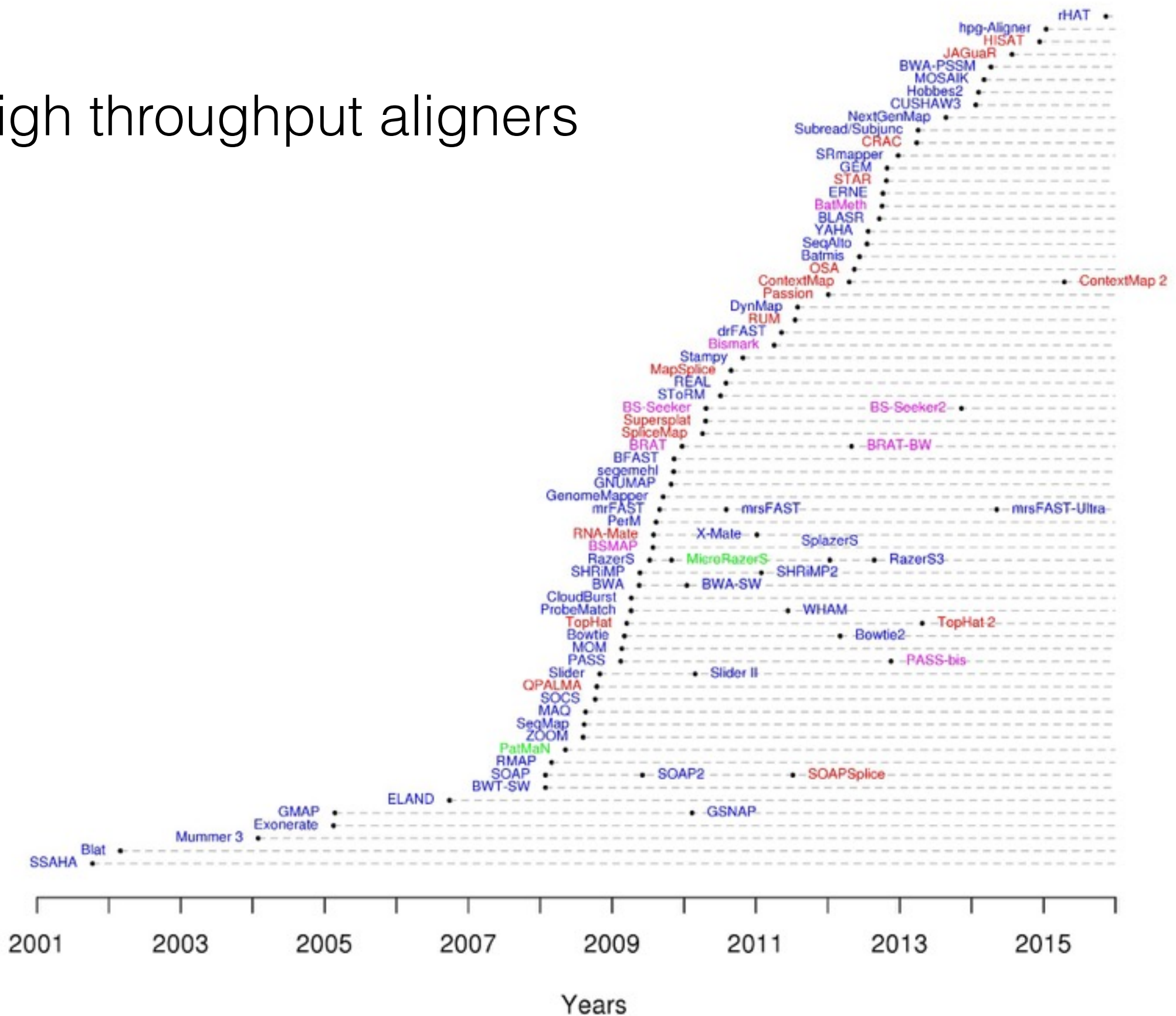
- Designed to identify homologous sequences.
- Hashed seed-extend algorithm
- First finds highly conserved or identical sequences which are then extended with a local alignment



BLAST

- 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

High throughput aligners

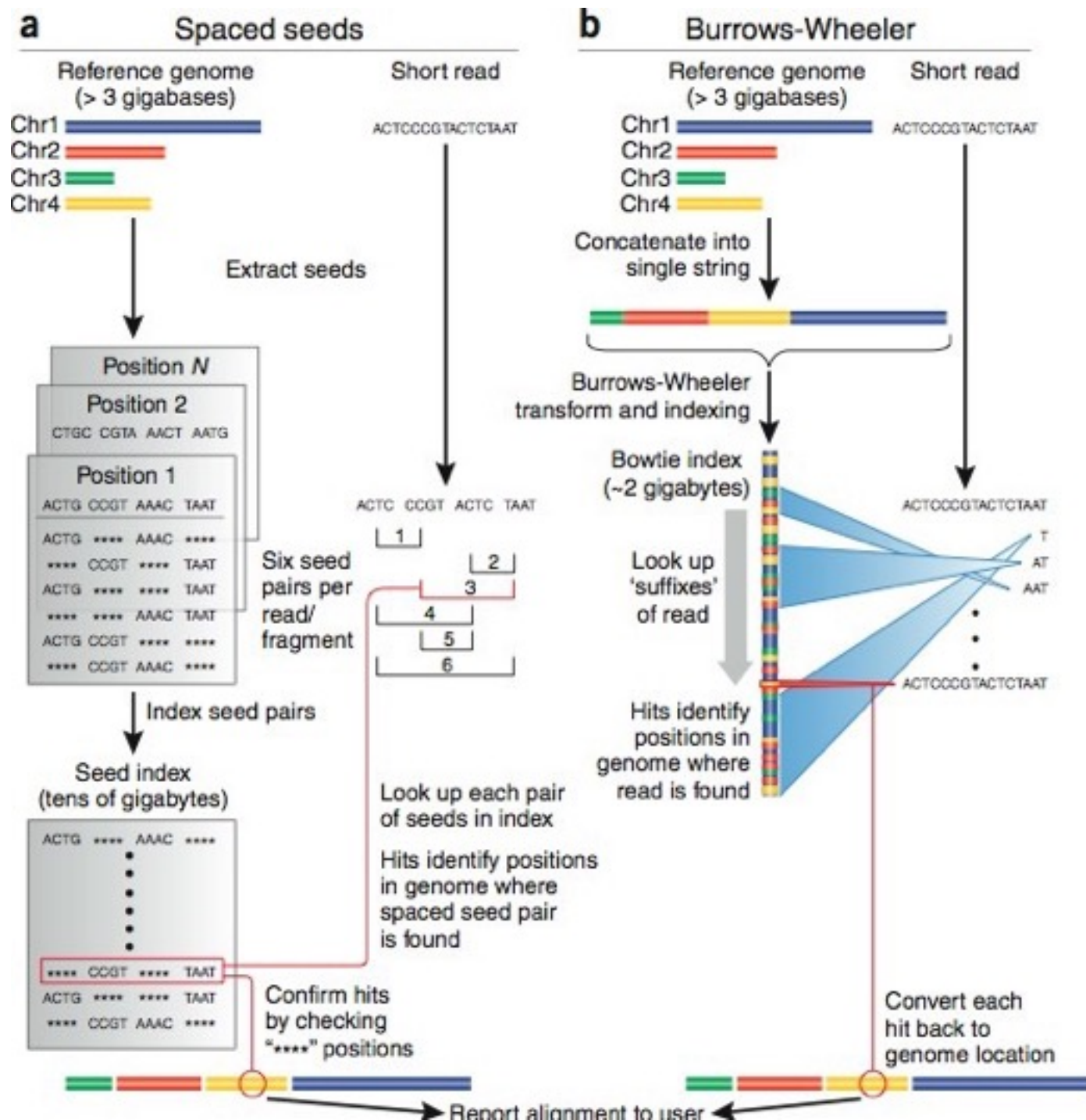


http://www.ebi.ac.uk/~nf/hts_mappers/

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

9bp seed

GATCGAAGG

9bp seed

ATTGATCAG

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

9bp seed

GATCGAAGG

9bp seed

ATTGATCAG

9bp seed

Here there is a match with at least one seed

Seed-extend algorithm

Reference sequence:

...GATCTCGATCGATGATCGTAGGATTGATCAGCTA...

Short read:

TAGATCGAT

9bp seed

GATCGAAGG

9bp seed

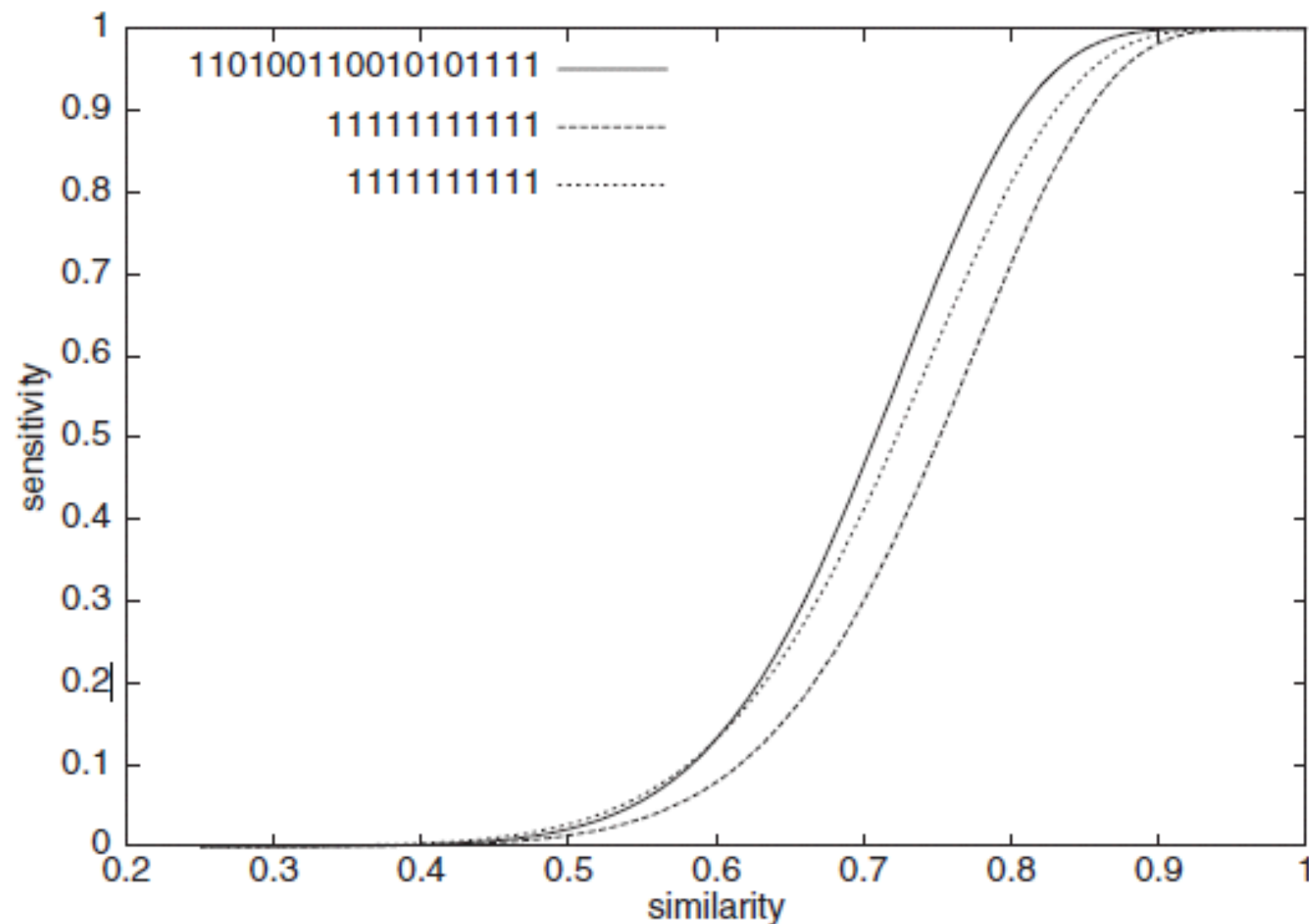
ATTGAGCAG

9bp seed

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

Spaced seeds

- To increase sensitivity we can use spaced-seeds:



Spaced seeds

- To increase sensitivity we can use spaced-seeds:

11111111

Consecutive seed template with **length** 9bp

GATAGCTAGCTAAT

Reference

AGCTAGCTA

Query

10101101011011

Consecutive seed template with **weight** 9bp

GATAGCTAGCTAAT

Reference

GATAGCGAGCTAAT

Query

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
<div>^BANANA </div>	<div>^BANANA </div> <div> ^BANANA</div> <div>A ^BANAN</div> <div>NA ^BANA</div> <div>ANA ^BAN</div> <div>NANA ^BA</div> <div>ANANA ^B</div> <div>BANANA ^</div>	<div>ANANA ^B</div> <div>ANA ^BAN</div> <div>A ^BANAN</div> <div>BANANA ^</div> <div>NANA ^BA</div> <div>NA ^BANA</div> <div>^BANANA </div> <div> ^BANANA</div>	<div>ANANA ^B</div> <div>ANA ^BAN</div> <div>A ^BANAN</div> <div>BANANA ^</div> <div>NANA ^BA</div> <div>NA ^BANA</div> <div>^BANANA </div> <div> ^BANANA</div>	<div>BNN^AA A</div>

Comparison

Hash referenced spaced seeds (NextGenMap)

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

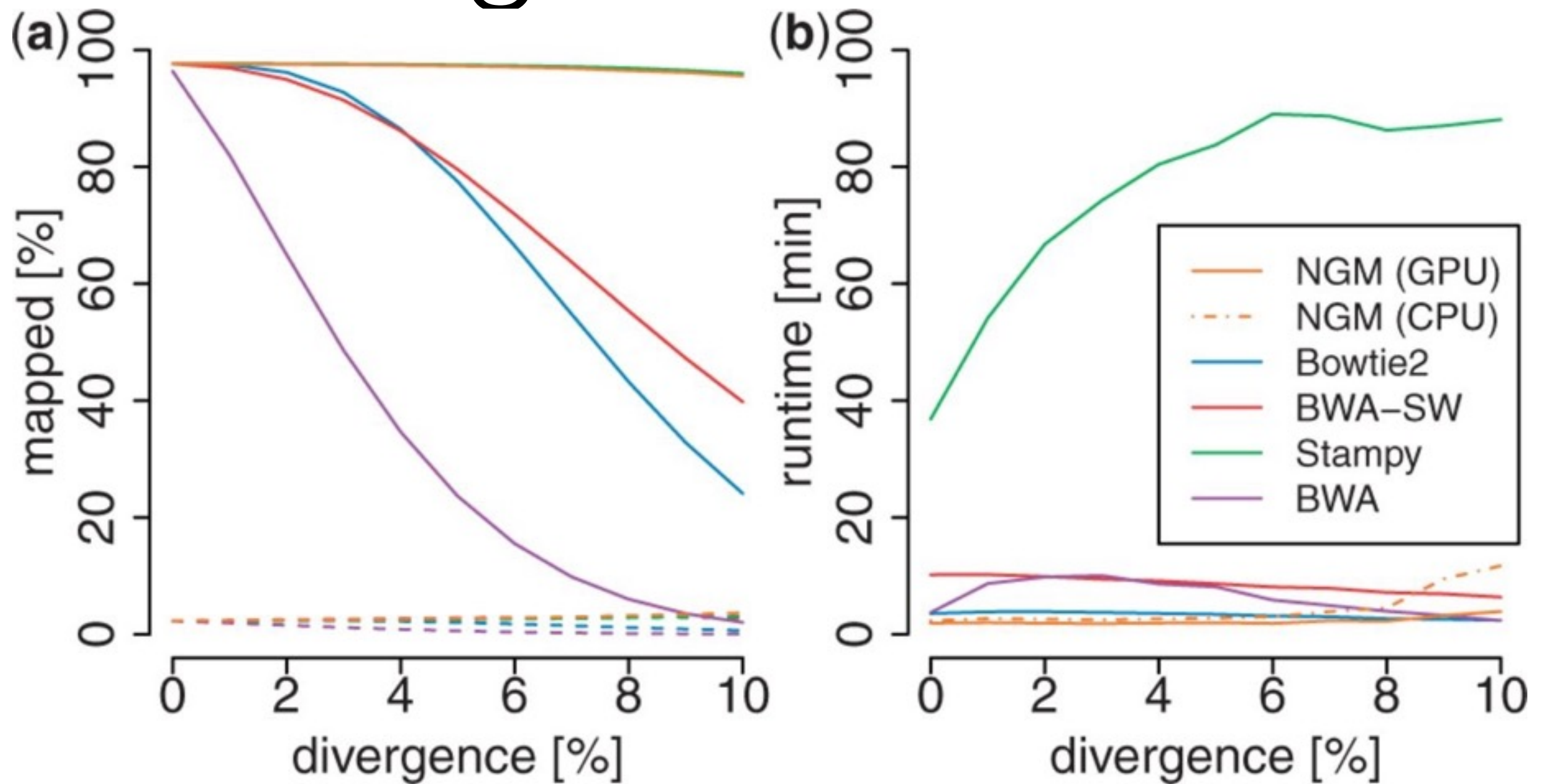
Suffix/Prefix Trie (BWA)

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

Popular short read aligners

Program	Algorithm	Speed	Accuracy in for divergent sequences
Bowtie2	FM-index	Very fast	Low
BWA	FM-index	Fast	Medium
Stampy	Hashing ref	Slow	High
Soap2	FM-index	Fast	Low
Novoalign	Hashing ref	Slow	High
NextGenMap	Hashing ref	Fast	High

Alignment stats



*From NextGenMap paper

Alignment choice

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

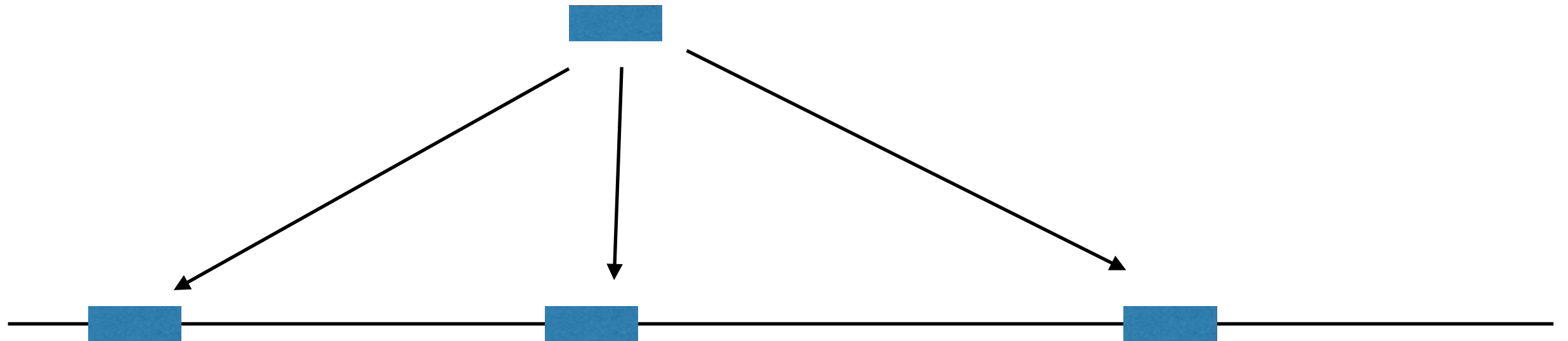
Other considerations

- PCR duplicates
- Multi-mapping reads
- Spliced-read mapping

PCR duplicates

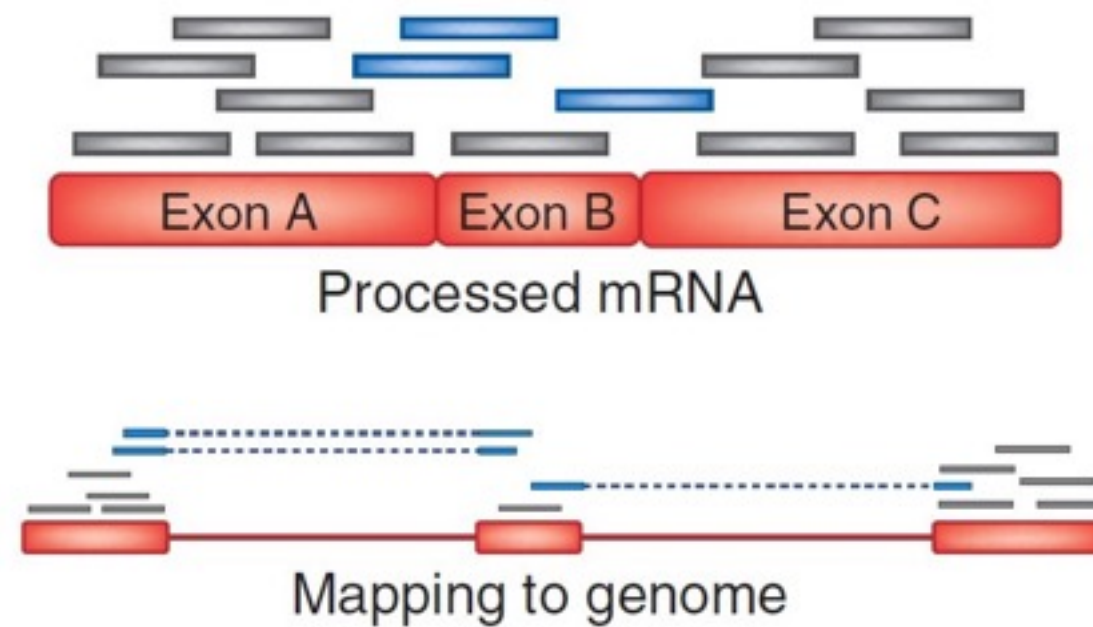
- Most library preps have at least one PCR amplification step
 - PCR can introduce errors and then sequencing multiple copies makes it seem like a real SNP
 - SAMtools and Picard can flag or remove these duplicates based on alignment location
 - Samples with same start and stop position are considered duplicates
 - Don't flag duplicates for GBS (set start and stop)

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

SAM format

Sort order

Reference sequence name and length

Read group information

Program information

```
@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

Read lines

SRR035022 163 chr16 59999 37 22D54M = 60102 179 CCAACCCAAC... >AAA=>?AA... XT:A:M XN:i:2 SM:i:37
<QNAME> <FLAG> <RNAME> <POS> <MAPQ> <CIGAR> <MRNM> <MPOS> <ISIZE> <SEQ> <QUAL> [<TAG>]

Mapping Quality

- $\text{MapQ} = Q_s = -10 \log_{10}(P)$
- P = probability that this mapping is NOT the correct one
- $\text{MapQ} = 0$ = equally likely to map somewhere else
- Different programs use different formulas for P