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

Scoring:
Matching letter: +1
Mismatch letter: -1
Inserting gap: -2

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

Total score = -1

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

Dynamic programming

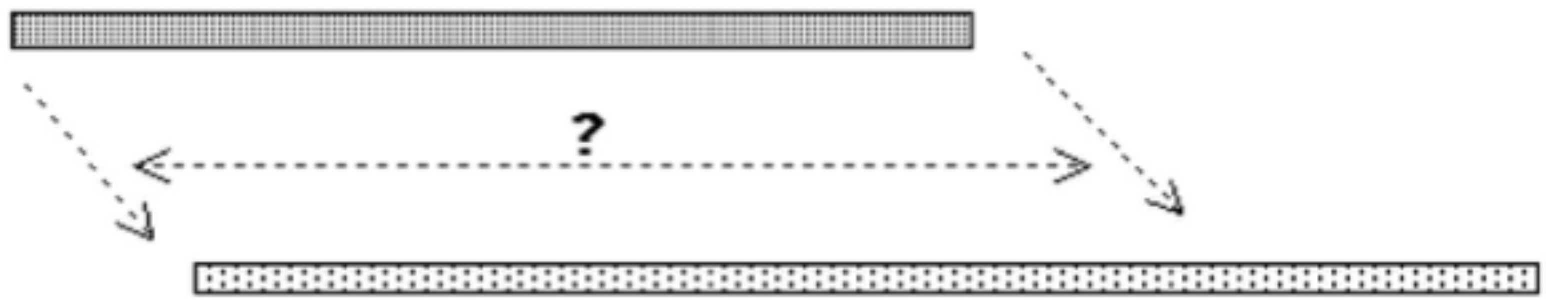
- Dynamic programming is a general programming technique.
- It structures a large search space into a succession of stages
    - The initial stage contains trivial solutions to sub-problems
    - Each partial solution in a later stage can be calculated by recurring a fixed number of partial solutions in an earlier stage
    - The final stage contains the overall solution

Global vs Local alignments

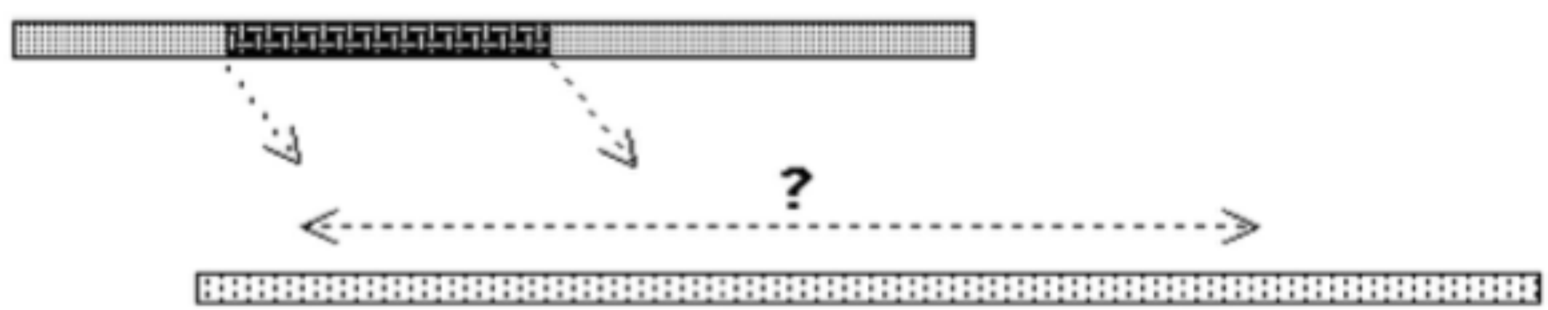
- Global alignment algorithms start at the beginning of two sequences and add gaps to each until the end of one is reached (Needleman-Wunsch).

- Local alignment algorithms finds 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 $F(i\text{-}1,j\text{-}1)$, $F(i\text{-}1,j)$ and $F(i,j\text{-}1)$ are known we can calculate $F(i,j)$
  - Three possibilities:
    - x_i and y_j are aligned, $F(i,j) = F(i\text{-}1,j\text{-}1) + s(x_i, y_j)$
    - x_i is aligned to a gap, $F(i,j) = F(i\text{-}1,j) - d$
    - y_j is aligned to a gap, $F(i,j) = F(i,j\text{-}1) - d$
      - 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

<u>Scoring system:</u>
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

<u>Scoring system:</u>

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

<u>Scoring system:</u>
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

<u>Scoring system:</u>

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

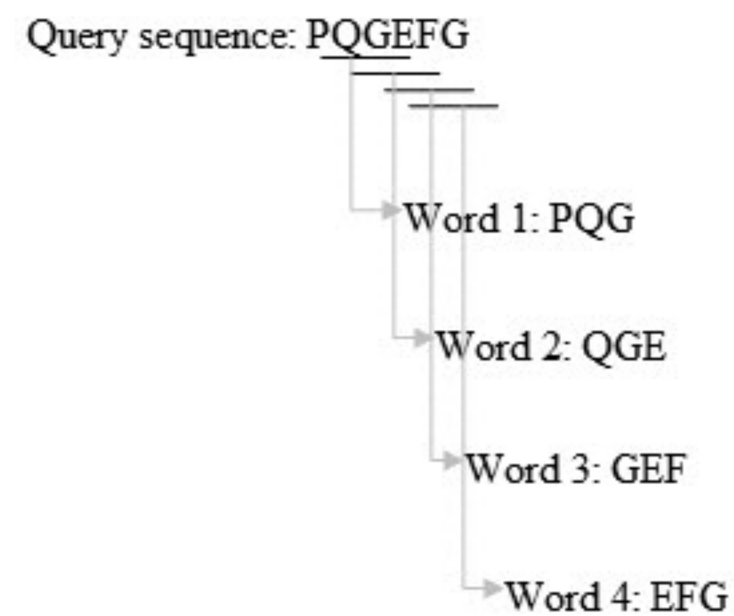
- Scoring systems:
  - Each symbol pairing is assigned a numerical value, based on a symbol comparison table.
    - nucleotides
    - amino acids (PAM, BLOSUM)
- Gap penalties:
  - Opening: The cost of introducing a gap.
  - Extension: The cost to elongate a gap.

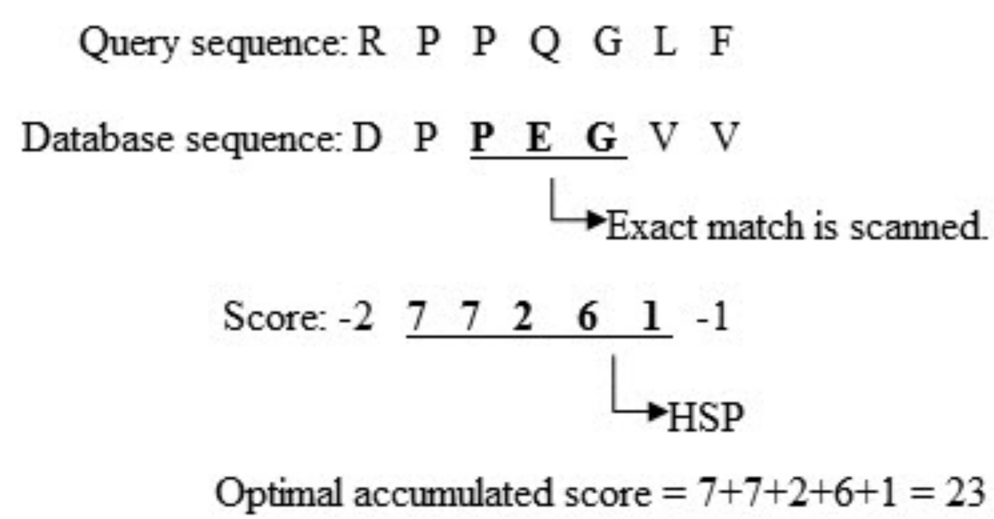
Gap penalties

- 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

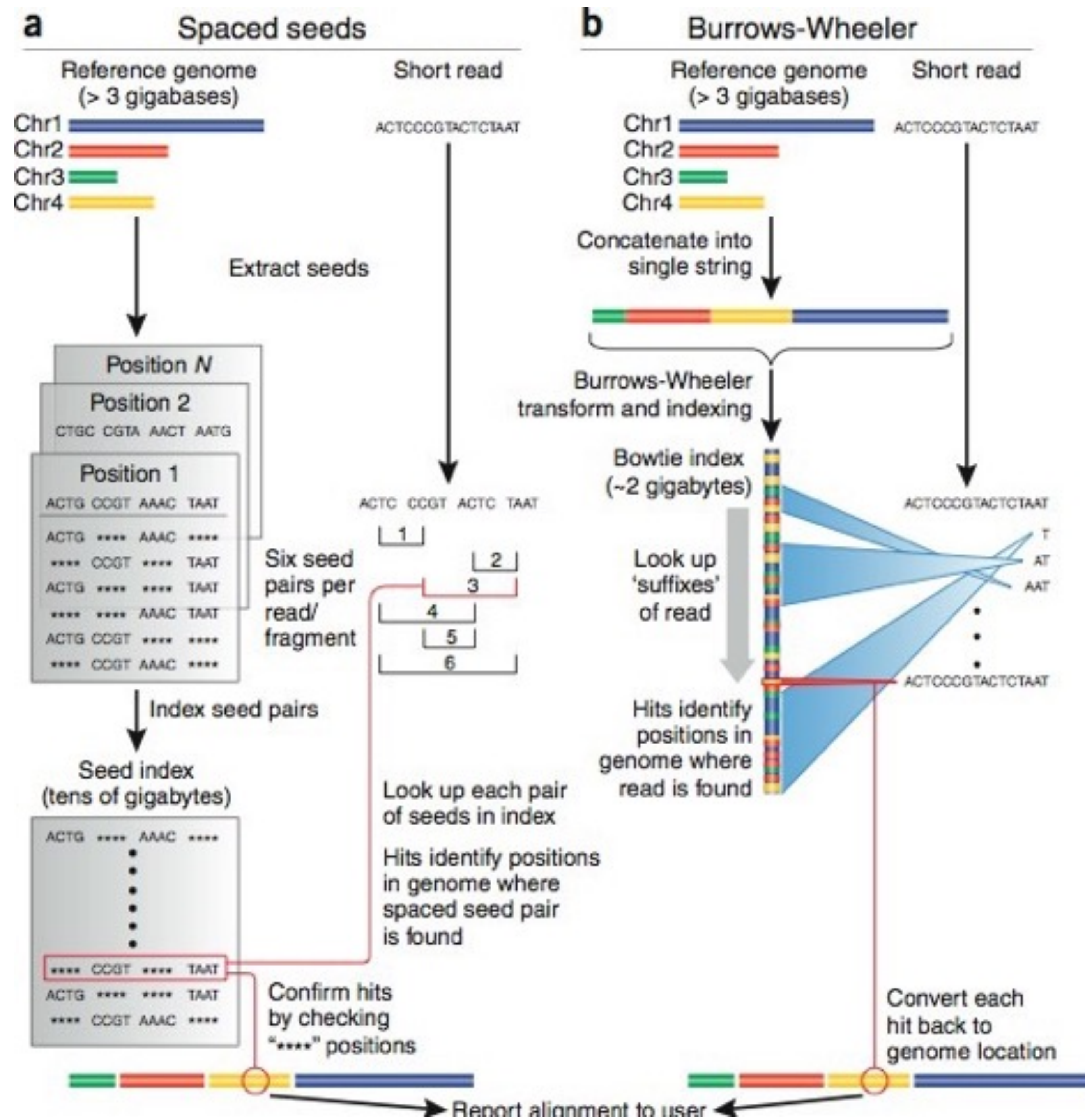
- Why not use BLAST for short read data?
  - Typically takes 0.1 to 1 second to search 1 sequence against a database
  - 60 million reads equates to 70 CPU days

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:

<u>seed</u> <u>->Extend with Smith-Waterman-></u>

…GATCTCGATCGATGATCGTAGGATTGATCAGCTA…

TCGATCGATGATCG<u>A</u>AGGATTGATCAG

Short read:

TCGATCGAT GATCG<u>A</u>AGG 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:

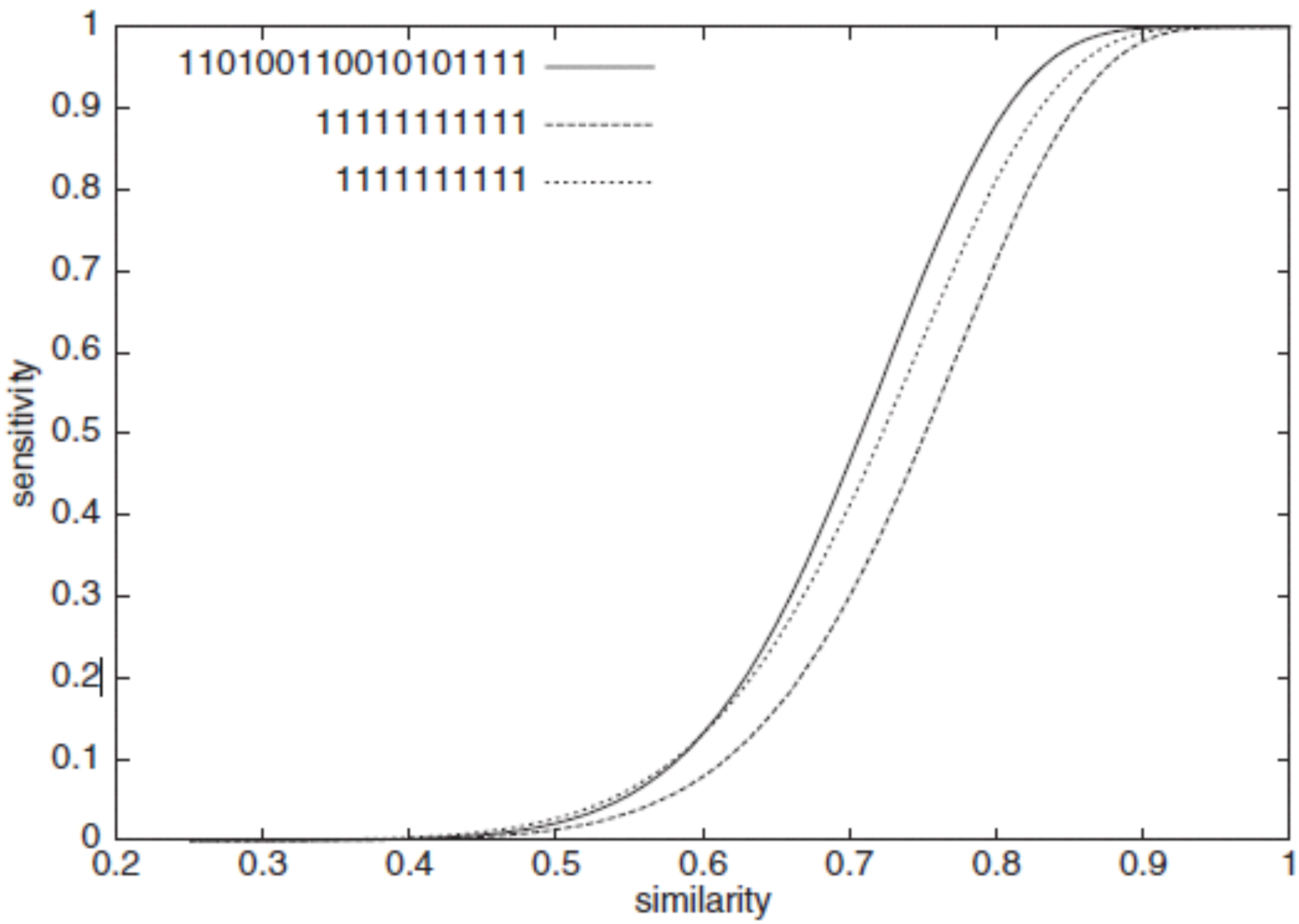
TAGATCGAT GATCGAAGG ATTGAGCAG

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        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

<table>
<tr><th colspan="5">Transformation</th></tr>
<tr><th>Input</th><th>All
Rotations</th><th>Sorting All Rows in Alphabetical
Order by their first letters</th><th>Taking
Last Column</th><th>Output
Last Column</th></tr>
<tr><td>^BANANA|</td><td>^BANANA|
|^BANANA
A|^BANAN
NA|^BANA
ANA|^BAN
NANA|^BA
ANANA|^B
BANANA|^</td><td>ANANA|^B
ANA|^BAN
A|^BANAN
BANANA|^
NANA|^BA
NA|^BANA
^BANANA|
|^BANANA</td><td>ANANA|^B
ANA|^BAN
A|^BANAN
BANANA|^
NANA|^BA
NA|^BANA
^BANANA|
|^BANANA</td><td>BNN^AA|A</td></tr>
</table>

Comparison

Hash referenced spaced seeds (NextGenMap)

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

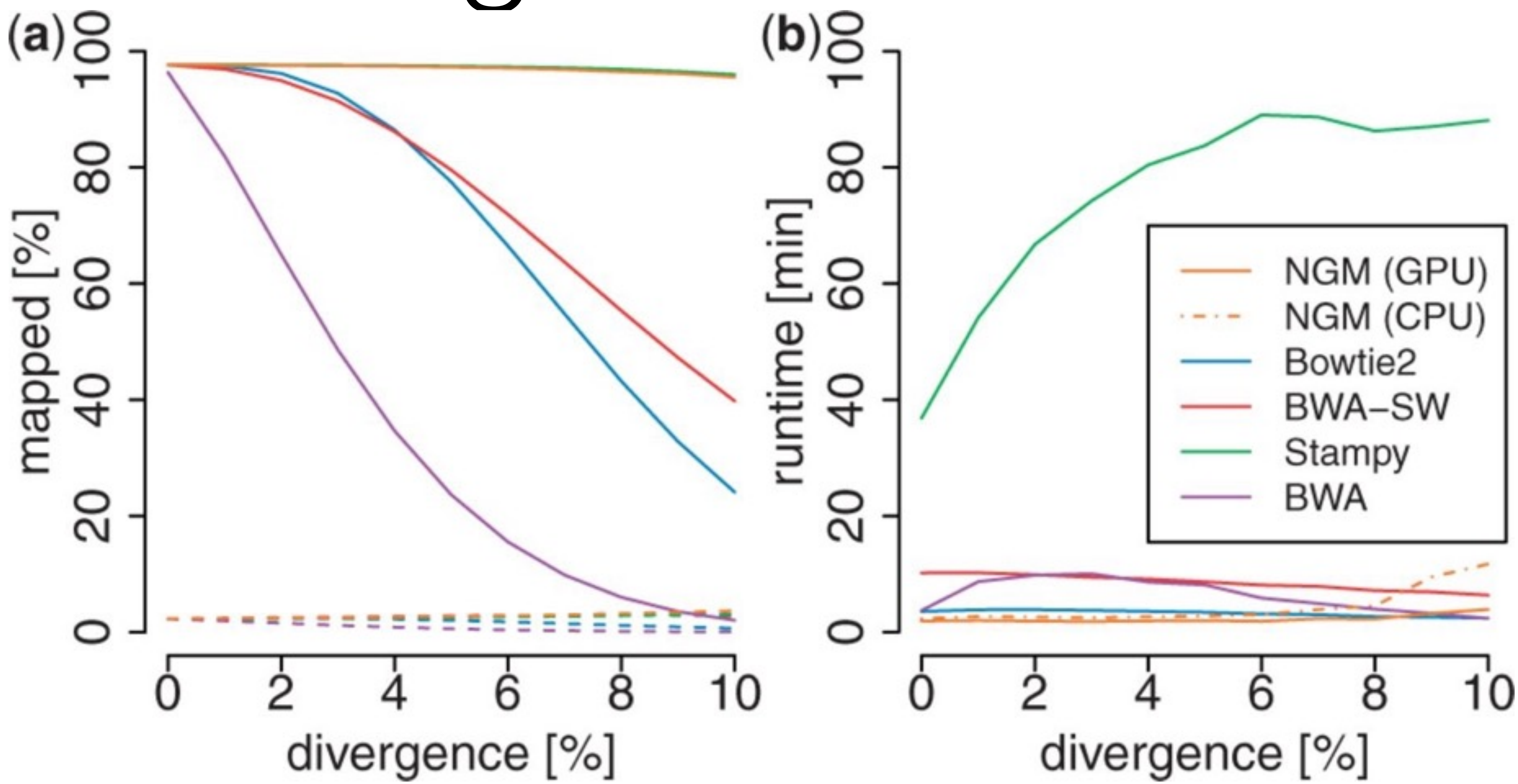
Suffix/Prefix Trie (BWA)

- 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?

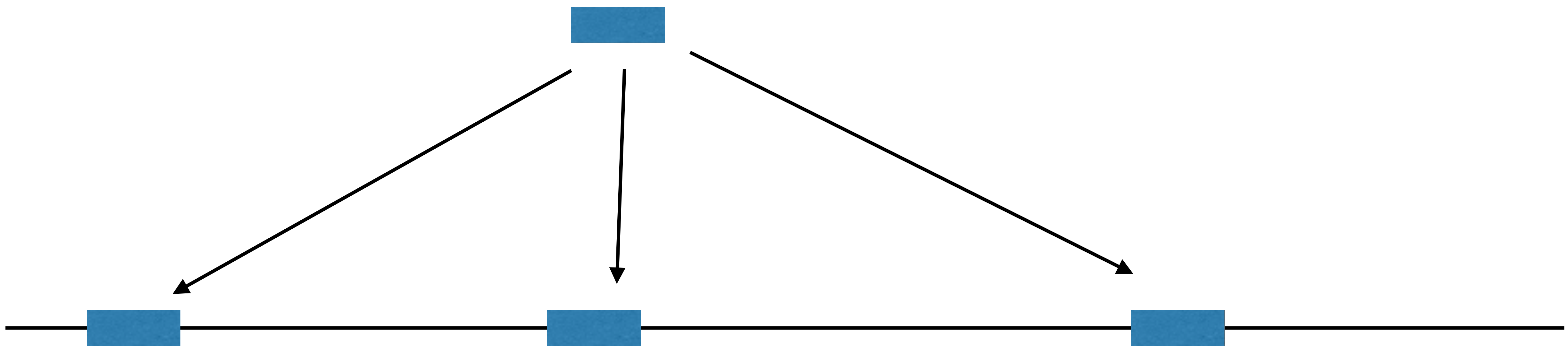
Other considerations

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

PCR duplicates

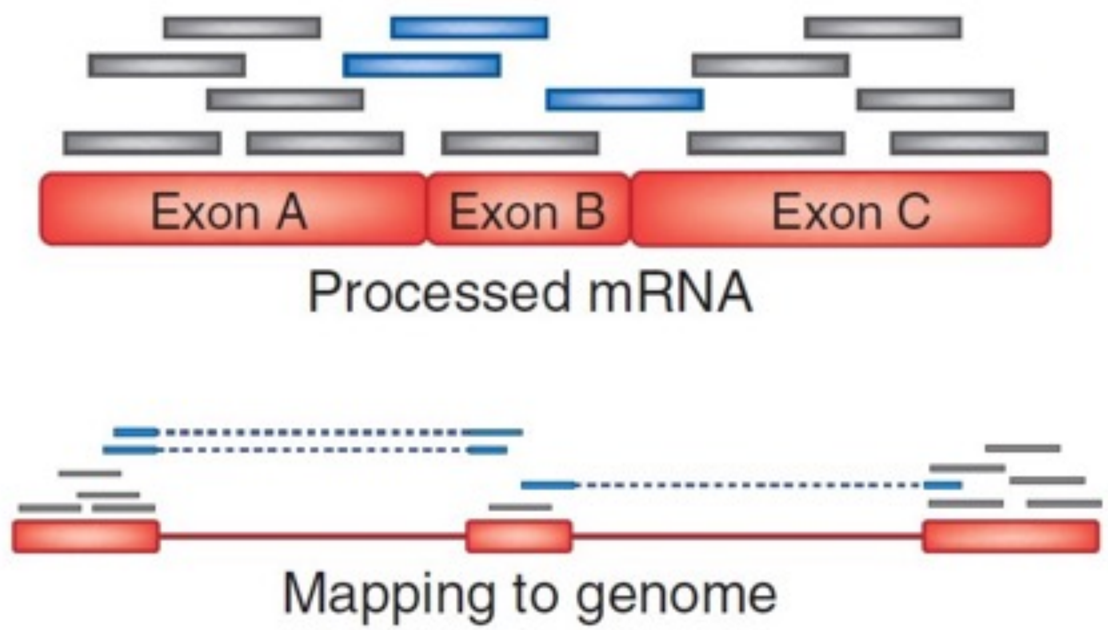
- Most library preps have at least one PCR amplification step
  - PCR can introduce errors and then sequencing multiple copies makes it seem like a real SNP
  - SAMtools and Picard can flag or remove these duplicates based on alignment location
    - Samples with same start and stop position are considered 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

```
@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 --
```

Program information

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

- 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