DTU

DTU Health Technology
Bioinformatics

Alignment

Gabriel Renaud
Associate Professor
Section of Bioinformatics
Technical University of Denmark
gabre@dtu.dk

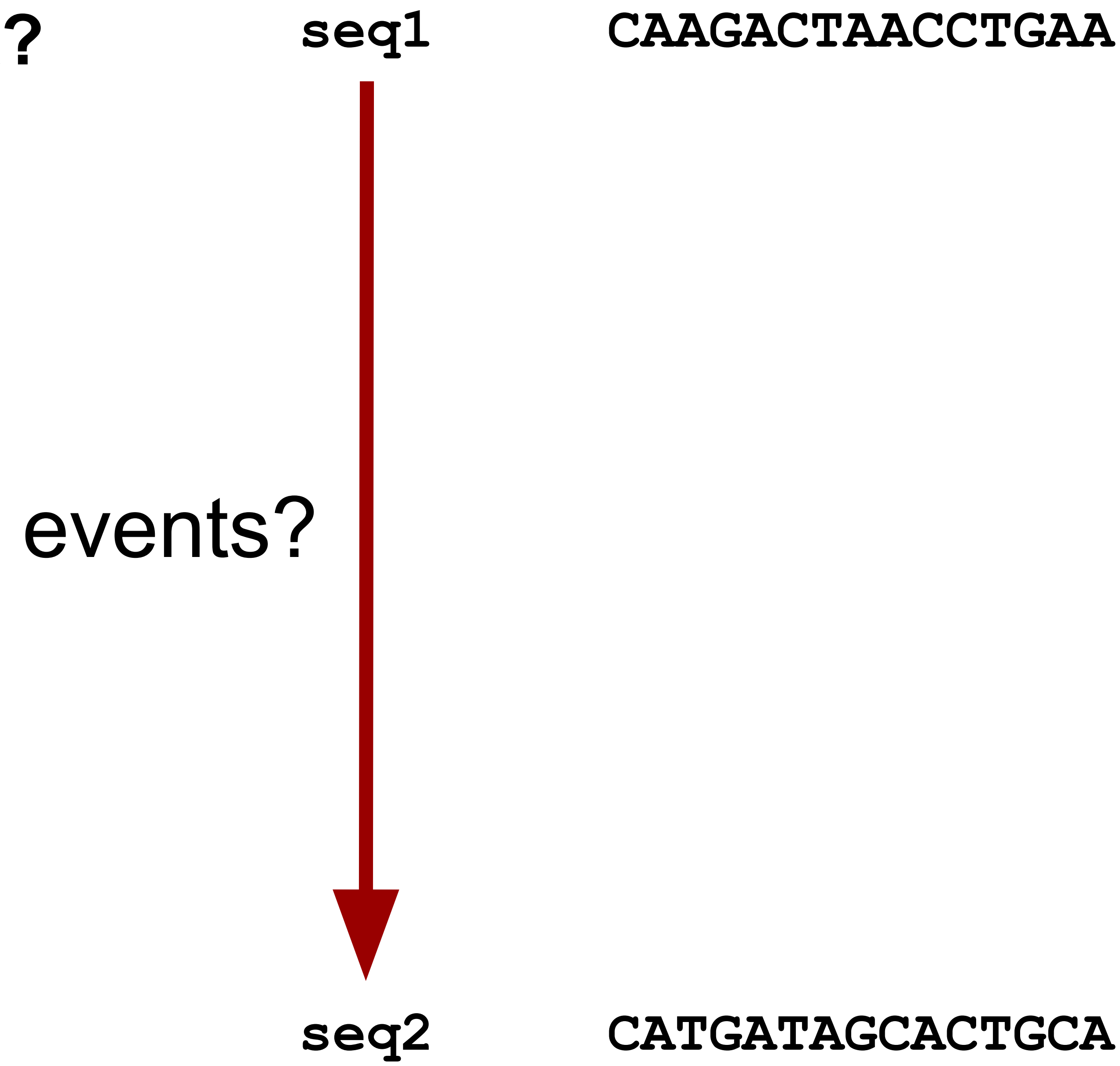
Menu

- Alignment approaches
- Burrows-Wheeler Transform
- More about coverage and depth
- Storing sequence alignments

What is an alignment?

Alignment = story

```
seq1:   CAAGACTAACCTGAA
seq2:   CATGATAGCACTGCA
```



seq1 CAAGACTAACCTGAA

events?

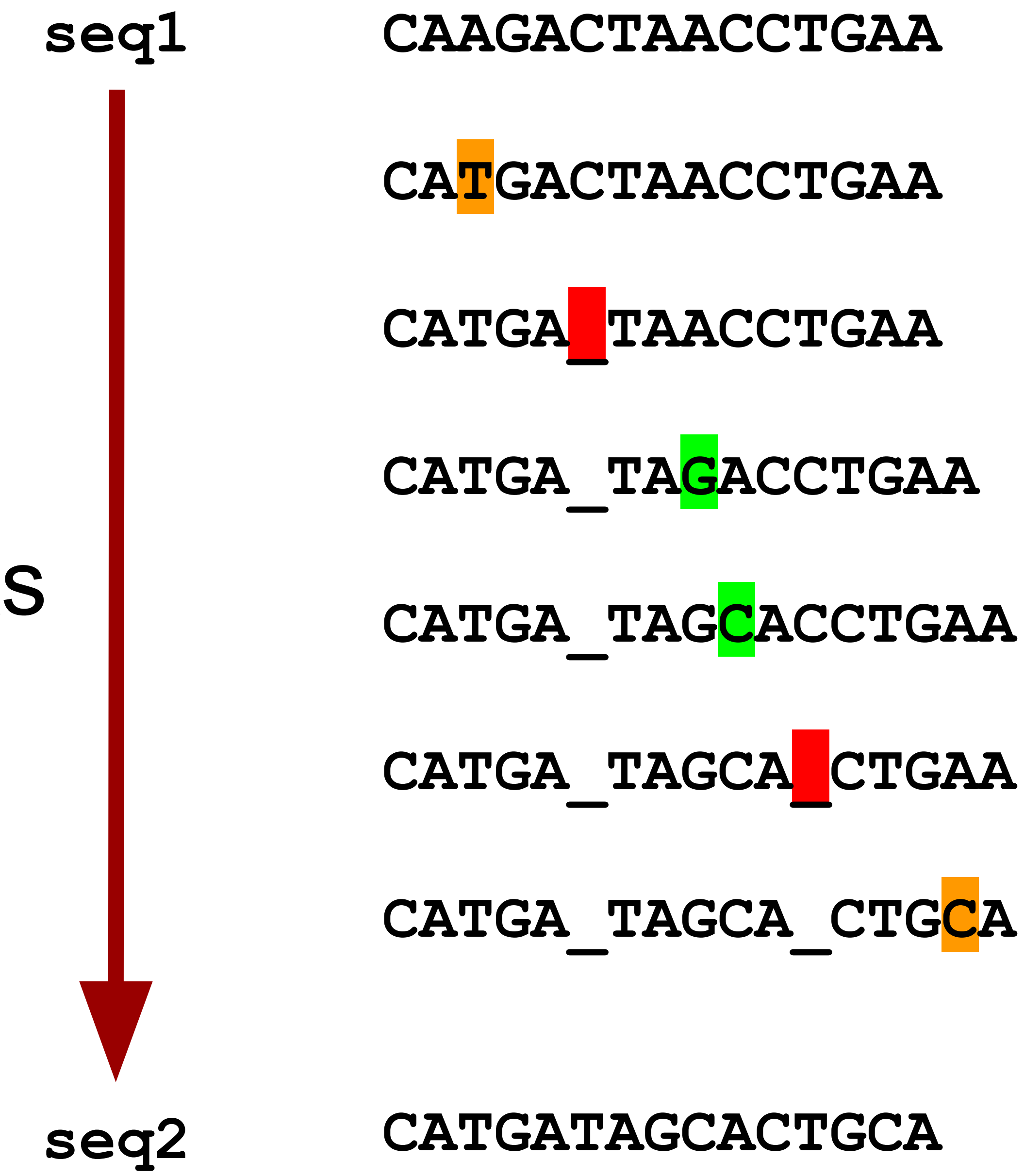
seq2 CATGATAGCACTGCA

What is an alignment?

Alignment = story

```
seq1:   CAAGACTAACCTGAA
seq2:   CATGATAGCACTGCA
```

6 events



```
seq1    CAAGACTAACCTGAA

        CATGACTAACCTGAA

        CATGA_TAACCTGAA

        CATGA_TAGACCTGAA

        CATGA_TAGCACCTGAA

        CATGA_TAGCA_CTGAA

        CATGA_TAGCA_CTGCA

seq2    CATGATAGCACTGCA
```

What is an alignment?

Alignment = story

```
seq1:   CAAGACTAACCTGAA
seq2:   CATGATAGCACTGCA
```

Needleman-Wunsch: best story?

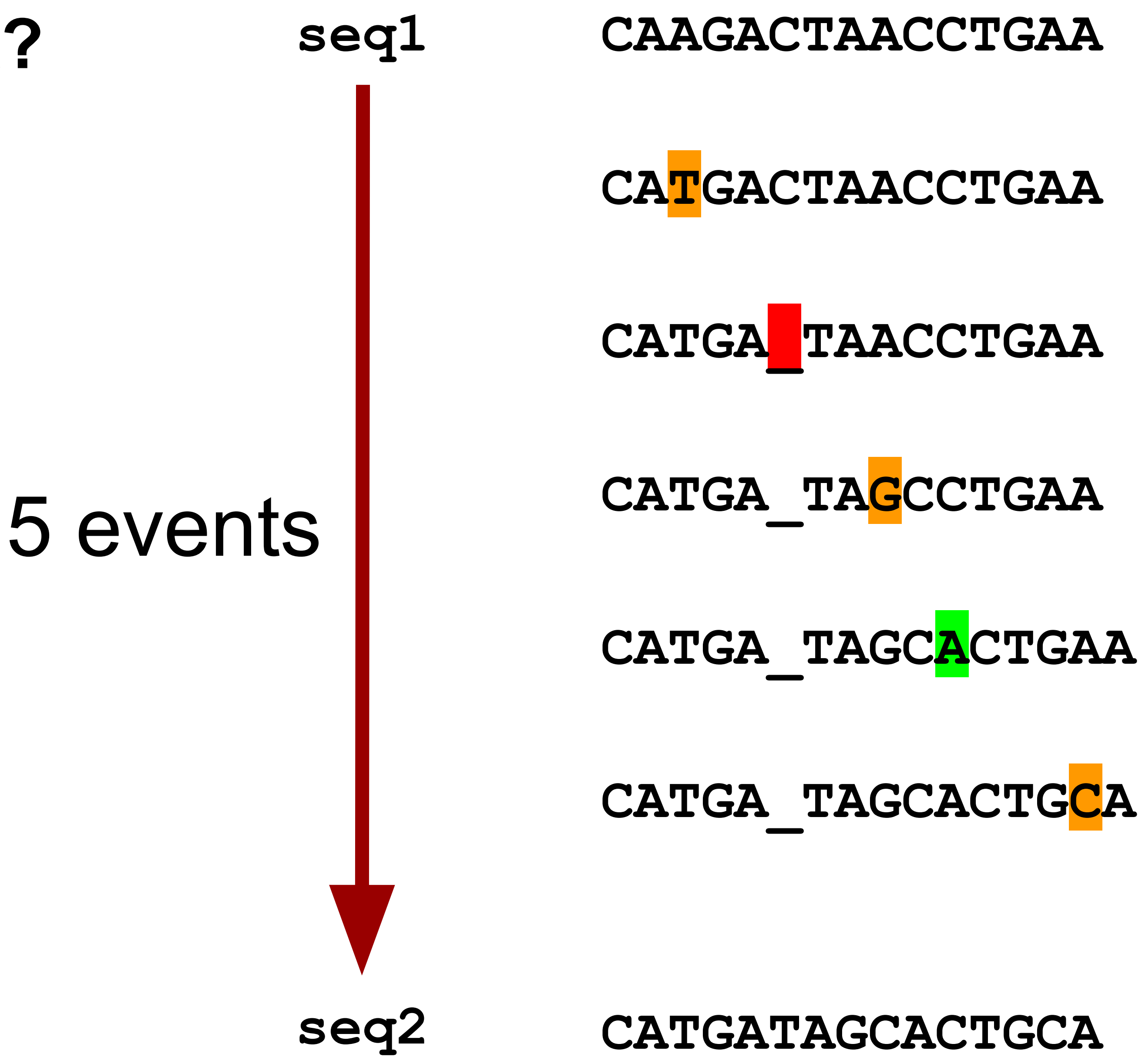
- Dynamic programming
- Find the best path in the matrix

D		C	A	T	G	A	T	A	G	C	A	C	T	G	C	A
	0	-2	-4	-6	-8	-10	-12	-14	-16	-18	-20	-22	-24	-26	-28	-30
C	-2	1	-1	-3	-5	-7	-9	-11	-13	-15	-17	-19	-21	-23	-25	-27
A	-4	-1	2	0	-2	-4	-6	-8	-10	-12	-14	-16	-18	-20	-22	-24
A	-6	-3	0	1	-1	-1	-3	-5	-7	-9	-11	-13	-15	-17	-19	-21
G	-8	-5	-2	-1	2	0	-2	-4	-4	-6	-8	-10	-12	-14	-16	-18
A	-10	-7	-4	-3	0	3	1	-1	-3	-5	-5	-7	-9	-11	-13	-15
C	-12	-9	-6	-5	-2	1	2	0	-2	-2	-4	-4	-6	-8	-10	-12
T	-14	-11	-8	-5	-4	-1	2	1	-1	-3	-3	-5	-3	-5	-7	-9
A	-16	-13	-10	-7	-6	-3	0	3	1	-1	-2	-4	-5	-4	-6	-6
A	-18	-15	-12	-9	-8	-5	-2	1	2	0	0	-2	-4	-6	-5	-5
C	-20	-17	-14	-11	-10	-7	-4	-1	0	3	1	1	-1	-3	-5	-6
C	-22	-19	-16	-13	-12	-9	-6	-3	-2	1	2	2	0	-2	-2	-4
T	-24	-21	-18	-15	-14	-11	-8	-5	-4	-1	0	1	3	1	-1	-3
G	-26	-23	-20	-17	-14	-13	-10	-7	-4	-3	-2	-1	1	4	2	0
A	-28	-25	-22	-19	-16	-13	-12	-9	-6	-5	-2	-3	-1	2	3	3
A	-30	-27	-24	-21	-18	-15	-14	-11	-8	-7	-4	-3	-3	0	1	4

What is an alignment?

Alignment = story

```
seq1:   CAAGACTAACCTGAA
seq2:   CATGATAGCACTGCA
```



```
seq1     CAAGACTAACCTGAA

         CATGACTAACCTGAA

         CATGA_TAACCTGAA

5 events CATGA_TAGCCTGAA

         CATGA_TAGCACTGAA

         CATGA_TAGCACTGCA

seq2     CATGATAGCACTGCA
```

What is an alignment?

Alignment = story

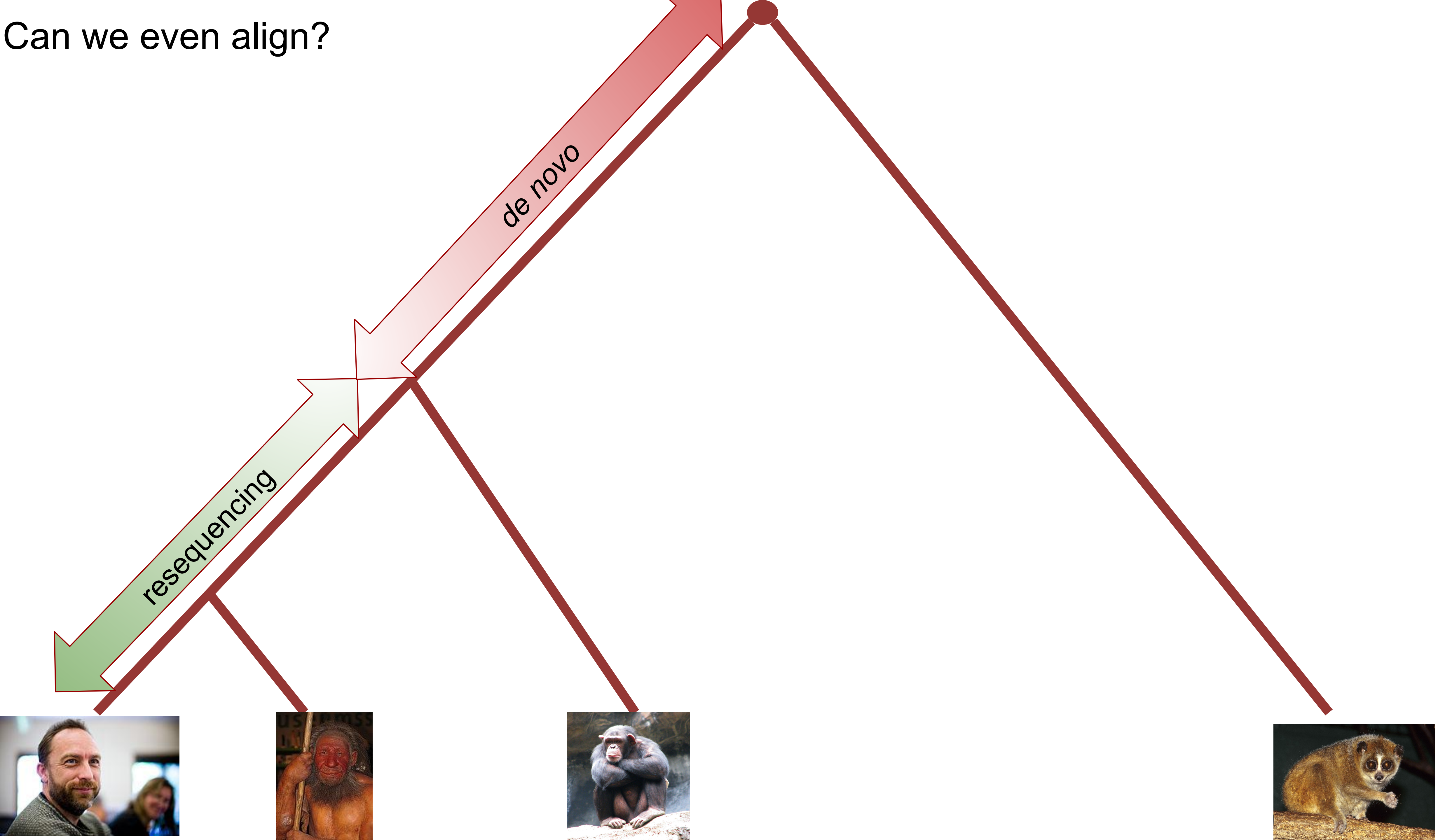
- 2 sequences can have a lot of alignments
- Not every alignment is equally likely
- Important to quantify the likelihood of seeing that alignment
- Be skeptical when you hear: “This is the alignment!”

What is an alignment?

Types of alignment:

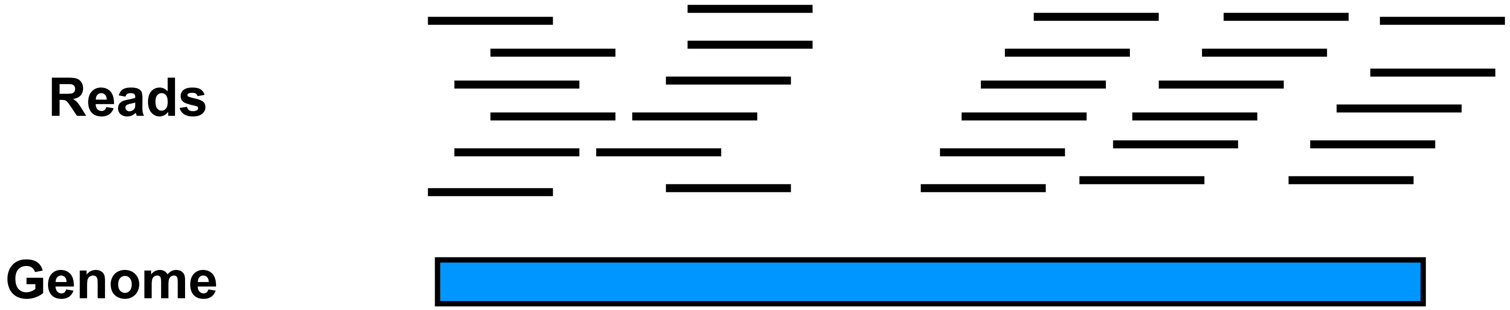
- “Short” read alignment
- Whole sequence alignment
- Whole genome/chromosome alignment
- Multiple sequence alignment

Can we even align?



Alignment/Mapping

- Assemble your reads by aligning them to a closely related reference genome
- High sequence similarity between individuals makes this possible



Sounds easy?

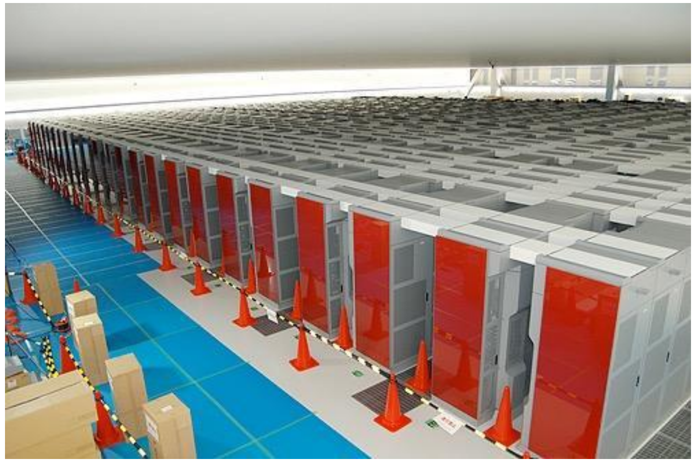
- Some pitfalls:
  - Divergence between sample and reference genome
  - Repeats in the genome
  - Recombination and re-arrangements
  - Poor reference genome quality
  - Read errors
  - Regions not in the ref. genome
  - Surprise sample

Simplest solution

- Exact string matches:
  (does not work)

```
Reference: ...ACGTGCGGACGCTGAACGTGA...
                  ||| |||||| ||
Read:             GCGCACGCTGTAC
```

- We need to allow mismatches/indels (Smith-Waterman, Needleman-Wunsch)
- One of the worlds fastest computer (*K computer - RIKEN*)
- 20M reads 100 nt reads vs. human genome ~ 1 month
- We search each read vs. the <u>entire</u> reference



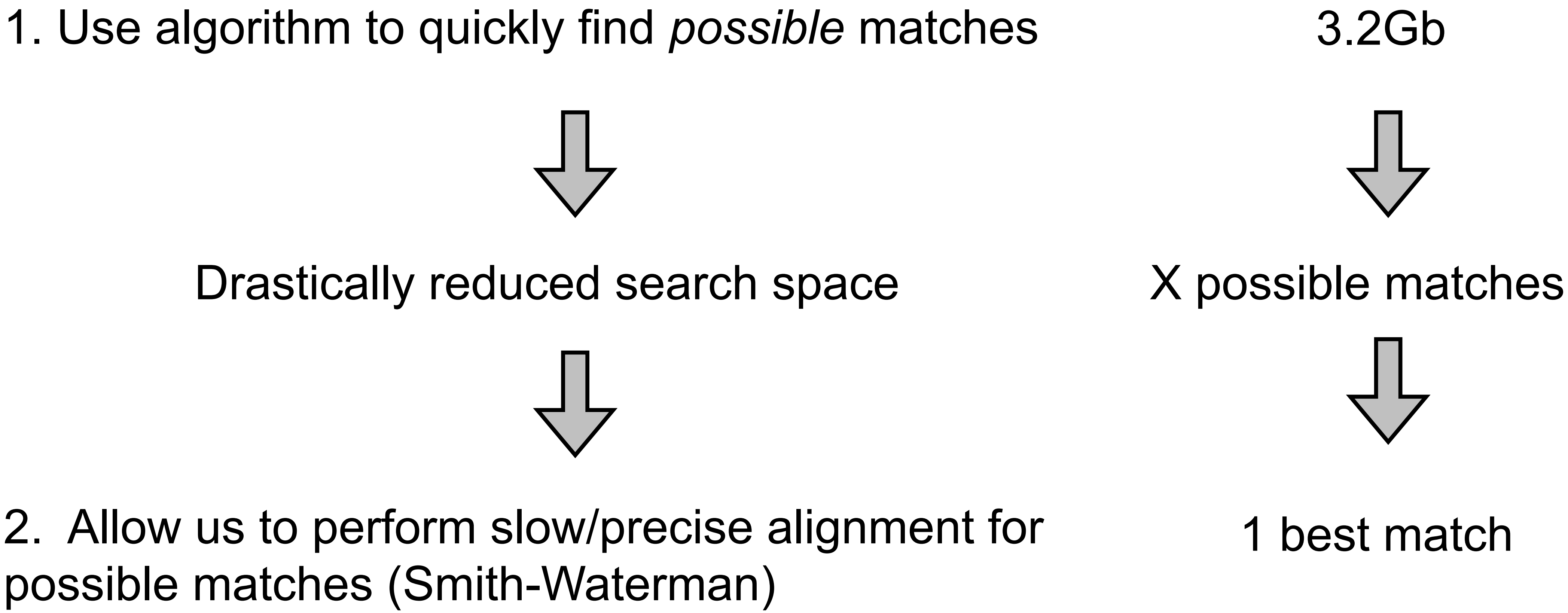
How about BLAST?

- Basic Local Alignment Search Tool
- Build list of “words” common to the reference+query

```
seq:   CAAGACTAACCTGAA
-----------------------
       CAAGACT
        AAGACTA
         AGACTAA
           ....
```

- Sensitive, great for finding remote homologs:
  - given a human protein, find the mouse one
- Way too slow for large number of short reads

Smart solution



1. Use algorithm to quickly find *possible* matches

Drastically reduced search space

2. Allow us to perform slow/precise alignment for possible matches (Smith-Waterman)

3.2Gb

X possible matches

1 best match

Hash based algorithms

Lookups in hashes are *fast!*

1. Index the reference using *k*-mers.
2. Search reads vs. hash *k*-mers
3. Perform alignment of entire read around seed
4. Report best alignment

Key → Value

.	.
.	.
.	.
ACTGCGTGTGA	Chr1_pos1234; Chr2_pos567
ACTGCGTGTGC	Chr7_posX
ACTGCGTGTGT	Chr7_posZ; ...
.	.
.	.
.	.

Also known as *Seed and extend*

Spaced seeds

- Key/*k*-mer is called a seed
- BLAST uses *k*=11 and all must be matches

```
11111111111
```

L = 11, 11 matches

- Smarter: Spaced seeds (only care about ‘1’ in seed, ‘0’ = wildcards)
  - Higher sensitivity
  - One can use several seeds

```
111010010100110111
```

L = 18, 11 matches

Multiple seeds & drawbacks

- One could require multiple short seeds
  - Instead of extending around each seed, extend around positions with several seed matches

- Drawbacks of hash-based approaches:
  - Lots(!) of RAM to keep index in memory (hg ~48Gb!)

Burrows-Wheeler Transform

- Hash based aligners require lots of memory and are only reasonable fast
- Can we make it better/faster?
- Burrows Wheeler Transformation (BWT)
- BWT was originally created for compression

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

```
T = AGGAGC$
```

1. Create all possible shifts of the string

(move first base to end)

```
0  AGGAGC$
```

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

```
T = AGGAGC$
```

1. Create all possible shifts of the string

(move first base to end)

```
0  AGGAGC$
1  GGAGC$A
```

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

```
T = AGGAGC$
```

1. Create all possible shifts of the string

(move first base to end)

```
0  AGGAGC$
1  GGAGC$A
2  GAGC$AG
```

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

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1. Create all possible shifts of the string

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```
0  AGGAGC$
1  GGAGC$A
2  GAGC$AG
3  AGC$AGG
```

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

T = AGGAGC$

0	AGGAGC$
1	GGAGC$A
2	GAGC$AG
3	AGC$AGG
4	GC$AGGA
5	C$AGGAG
6	$AGGAGC

1. Create all possible shifts of the string

(move first base to end)

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

T = AGGAGC$

0	AGGAGC$
1	GGAGC$A
2	GAGC$AG
3	AGC$AGG
4	GC$AGGA
5	C$AGGAG
6	$AGGAGC

2. Sort the strings lexicographically to create BWT matrix and Suffix Array

$	A	G	G	A	G	C
A	G	C	$	A	G	G
A	G	G	A	G	C	$
C	$	A	G	G	A	G
G	A	G	C	$	A	G
G	C	$	A	G	G	A
G	G	A	G	C	$	A

BWT matrix

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

T = AGGAGC$

BWT(T) = CG$GGAA

$	A	G	G	A	G	C
A	G	C	$	A	G	G
A	G	G	A	G	C	$
C	$	A	G	G	A	G
G	A	G	C	$	A	G
G	C	$	A	G	G	A
G	G	A	G	C	$	A

BWT matrix

BWT: Create index

Genome

Marks end-of-string, lexicographically smallest

```
T = AGGAGC$
```

- Reversible
- BTW(T) is easier to compress than T due to repeated characters tend to cluster ex:

```
BWT(T)= CG$GGAA
```

```
Ringeren_I_Ringe_ringer_ringere_end_ringeren_ringer_i_Ringsted$
$d__ _nIiernerdenrgtrr_gggggnnnnnnn_RrrrRrReeeiiiiiiieeeee____gs
```

- try bzip2

BWT: *T*-rank

T = AGGAGC$

T-ranking:

# of times the base occurred previously in T

$A_0 \; G_0 \; G_1 \; A_1 \; G_2 \; C_0 \; \$$

F						*L*
$	A	G	G	A	G	C
A	G	C	$	A	G	G
A	G	G	A	G	C	$
C	$	A	G	G	A	G
G	A	G	C	$	A	G
G	C	$	A	G	G	A
G	G	A	G	C	$	A

BWT: *T*-rank

T = AGGAGC$

T-ranking: # of times the base occurred previously in T

A_0 G_0 G_1 A_1 G_2 C_0 $

F						*L*
$	A_0	G_0	G_1	A_1	G_2	C_0
A_1	G_2	C_0	$	A_0	G_0	G_1
A_0	G_0	G_1	A_1	G_2	C_0	$
C_0	$	A_0	G_0	G_1	A_1	G_2
G_1	A_1	G_2	C_0	$	A_0	G_0
G_2	C_0	$	A_0	G_0	G_1	A_1
G_0	G_1	A_1	G_2	C_0	$	A_0

Notice that individual base-rank is the same in *F* and *L*

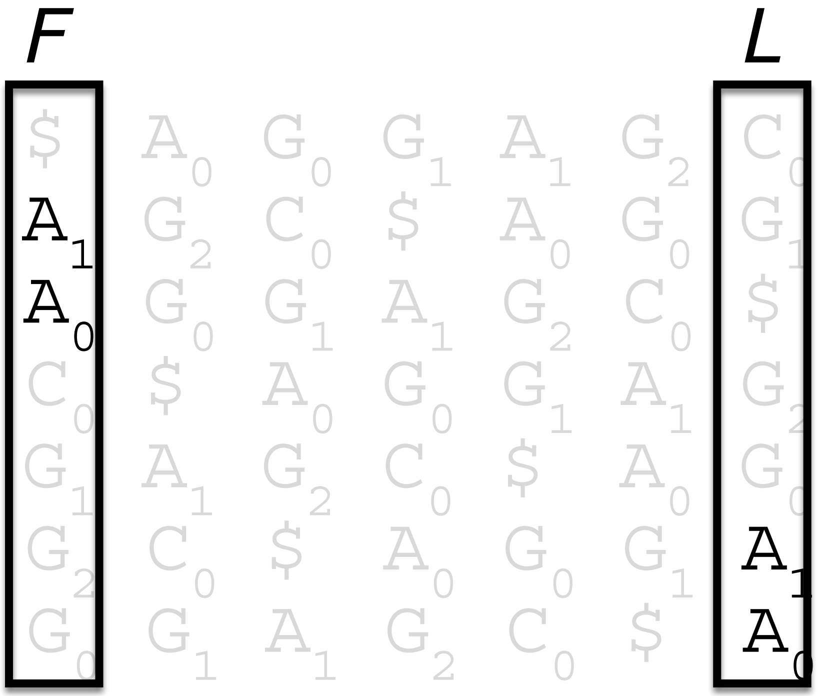
➡ Rank will always be the same in *F* and *L*

BWT: *T*-rank

T = AGGAGC$

T-ranking: # of times the base occurred previously in T

A_0 G_0 G_1 A_1 G_2 C_0 $



F … *L*

\$ A_0 G_0 G_1 A_1 G_2 C_0

A_1 G_2 C_0 \$ A_0 G_0 G_1

A_0 G_0 G_1 A_1 G_2 C_0 \$

C_0 \$ A_0 G_0 G_1 A_1 G_2

G_1 A_1 G_2 C_0 \$ A_0 G_0

G_2 C_0 \$ A_0 G_0 G_1 A_1

G_0 G_1 A_1 G_2 C_0 \$ A_0

Notice that individual base-rank is the same in *F* and *L*

➡ Rank will always be the same in *F* and *L*

BWT: *T*-rank

T = AGGAGC$

T-ranking: # of times the base occurred previously in T

A_0 G_0 G_1 A_1 G_2 C_0 $

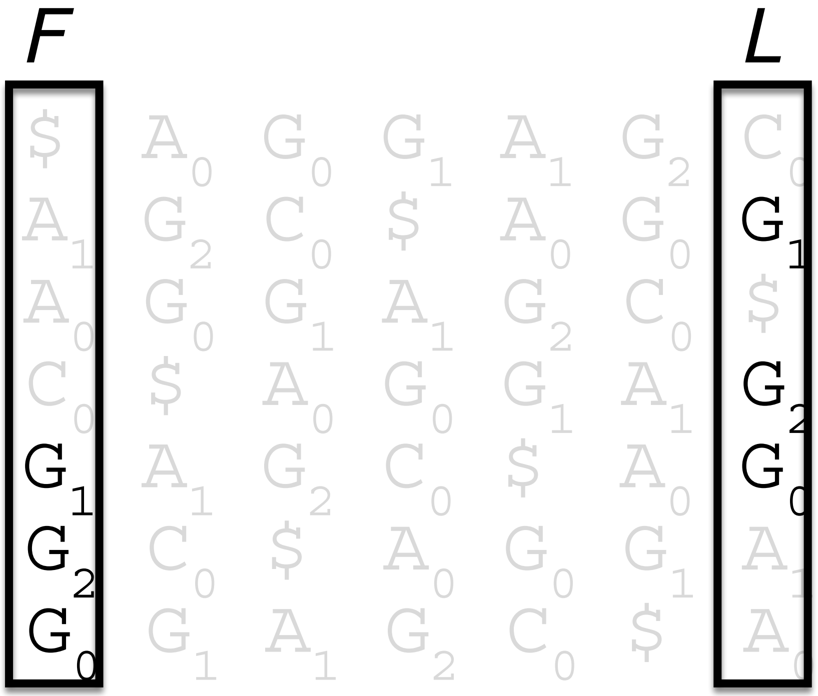
F						*L*
$	A_0	G_0	G_1	A_1	G_2	C_0
A_1	G_2	C_0	$	A_0	G_0	G_1
A_0	G_0	G_1	A_1	G_2	C_0	$
C_0	$	A_0	G_0	G_1	A_1	G_2
G_1	A_1	G_2	C_0	$	A_0	G_0
G_2	C_0	$	A_0	G_0	G_1	A_1
G_0	G_1	A_1	G_2	C_0	$	A_0

Notice that individual base-rank is the same in *F* and *L*

➡ Rank will always be the same in *F* and *L*

BWT: *T*-rank

Why does this generalize?



F						*L*
\$	A_0	G_0	G_1	A_1	G_2	C_0
A_1	G_2	C_0	\$	A_0	G_0	G_1
A_0	G_0	G_1	A_1	G_2	C_0	\$
C_0	\$	A_0	G_0	G_1	A_1	G_2
G_1	A_1	G_2	C_0	\$	A_0	G_0
G_2	C_0	\$	A_0	G_0	G_1	A_1
G_0	G_1	A_1	G_2	C_0	\$	A_0

BWT: *T*-rank

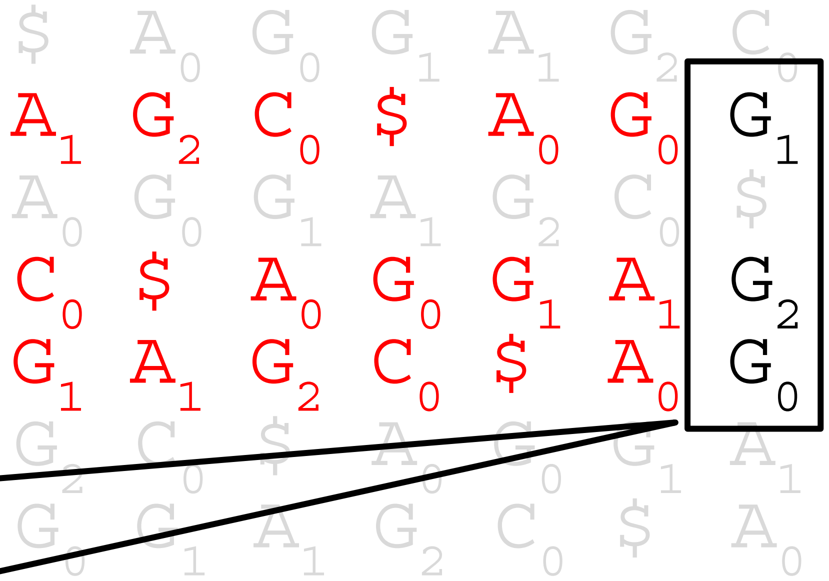
Why does this generalize?

F						*L*
$\$$	A_{0}	G_{0}	G_{1}	A_{1}	G_{2}	C_{0}
A_{1}	G_{2}	C_{0}	$\$$	A_{0}	G_{0}	G_{1}
A_{0}	G_{0}	G_{1}	A_{1}	G_{2}	C_{0}	$\$$
C_{0}	$\$$	A_{0}	G_{0}	G_{1}	A_{1}	G_{2}
G_{1}	A_{1}	G_{2}	C_{0}	$\$$	A_{0}	G_{0}
G_{2}	C_{0}	$\$$	A_{0}	G_{0}	G_{1}	A_{1}
G_{0}	G_{1}	A_{1}	G_{2}	C_{0}	$\$$	A_{0}

We are all the same letter, our order is determined by what is right of us (blue).

BWT: *T*-rank

Why does this generalize?

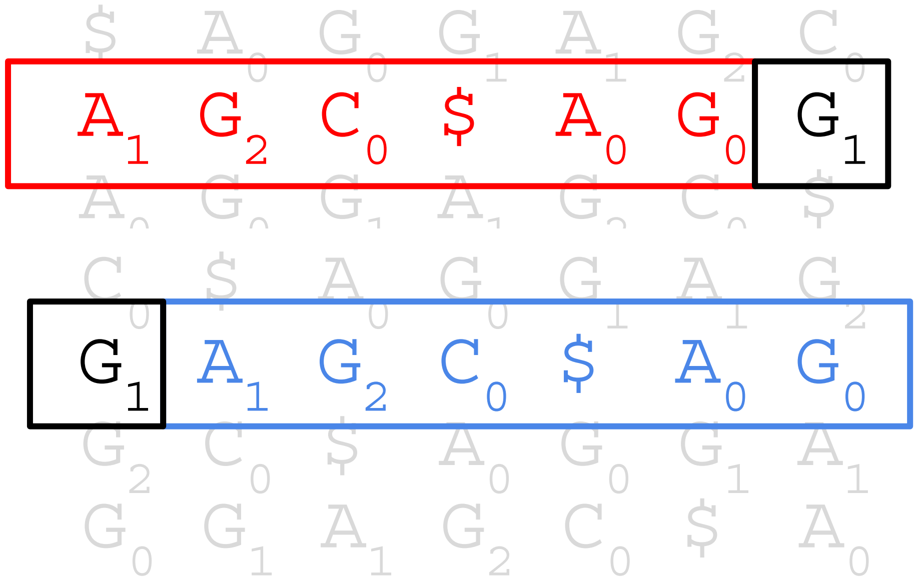


$	A_0	G_0	G_1	A_1	G_2	C_0
A_1	G_2	C_0	$	A_0	G_0	G_1
A_0	G_0	G_1	A_1	G_2	C_0	$
C_0	$	A_0	G_0	G_1	A_1	G_2
G_1	A_1	G_2	C_0	$	A_0	G_0
G_2	C_0	$	A_0	G_0	G_1	A_1
G_0	G_1	A_1	G_2	C_0	$	A_0

We are all the same letter, our order is determined by what is left of us (red).

BWT: *T*-rank

Why does this generalize?



$\$\ A_0\ G_0\ G_1\ A_1\ G_2\ C_0$

$A_1\ G_2\ C_0\ \$\ A_0\ G_0\ G_1$

$A_0\ G_0\ G_1\ A_1\ G_2\ C_0\ \$$

$C_0\ \$\ A_0\ G_0\ G_1\ A_1\ G_2$

$G_1\ A_1\ G_2\ C_0\ \$\ A_0\ G_0$

$G_2\ C_0\ \$\ A_0\ G_0\ G_1\ A_1$

$G_0\ G_1\ A_1\ G_2\ C_0\ \$\ A_0$

- The string left (red) of the G1 and right (blue) of G1 are identical
- They are sorted
- Therefore the order is the same

BWT: *T*-rank

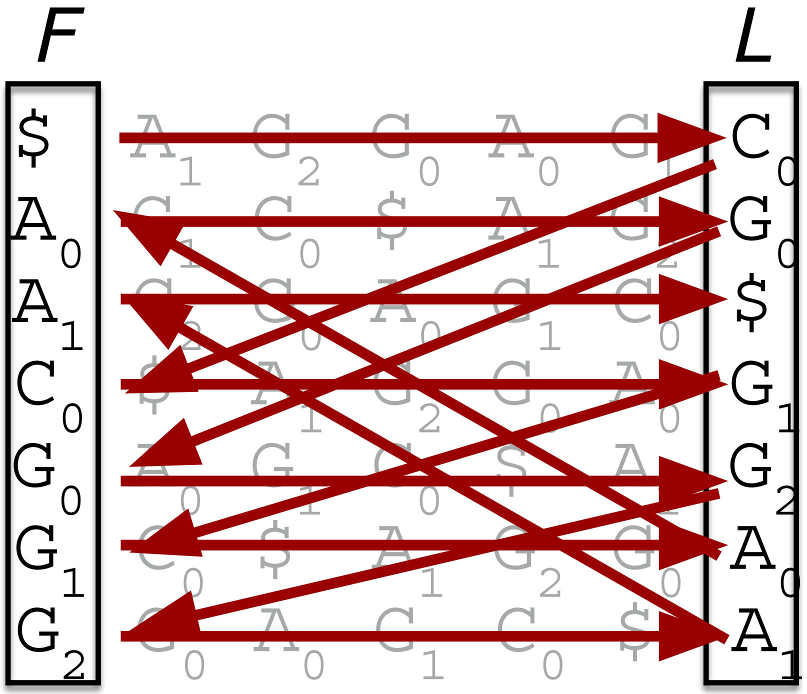
How to reverse the BTW back the original string *T* ?

T-rank

F						*L*
$\$$	A_1	G_2	G_0	A_0	G_1	C_0
A_0	G_1	C_0	$\$$	A_1	G_2	G_0
A_1	G_2	G_0	A_0	G_1	C_0	$\$$
C_0	$\$$	A_1	G_2	G_0	A_0	G_1
G_0	A_0	G_1	C_0	$\$$	A_1	G_2
G_1	C_0	$\$$	A_1	G_2	G_0	A_0
G_2	G_0	A_0	G_1	C_0	$\$$	A_1

BWT is reversible

LF-mapping: LF can be used to recreate the original genome



$C_0G_1A_0G_0G_2A_1\$$

Reversed:

$A_1G_2G_0A_0G_1C_0$

T = AGGAGC$

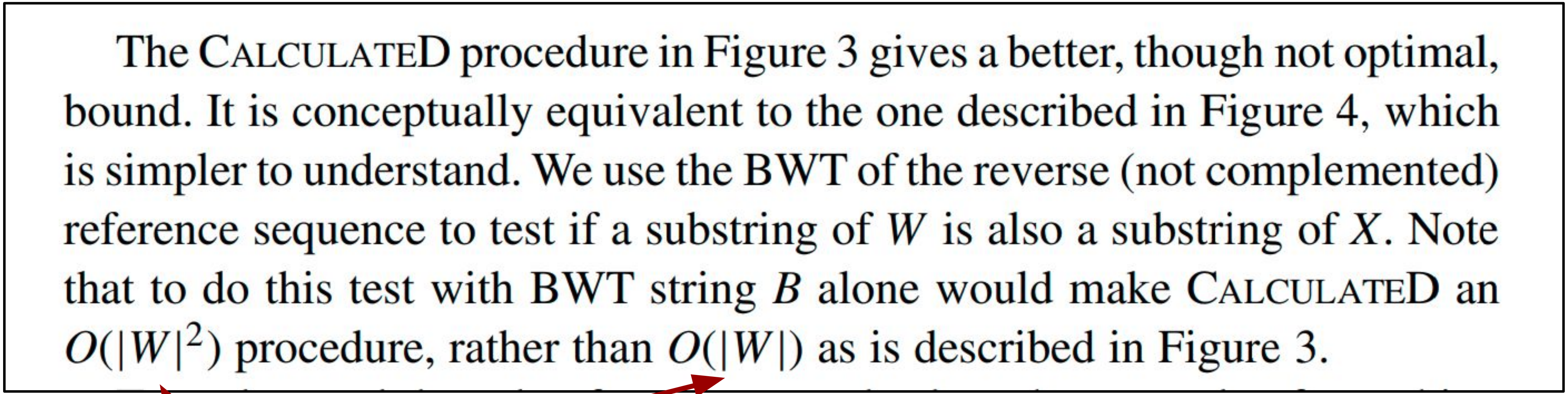
F can be represented = 2x A, 1x C, 3x G we need |Σ| integers

We therefore only need to store *L*

Why are we talking about BTW for alignments?

- In 1994, Michael Burrows and David Wheeler created the Burrows-Wheeler Transform (BWT)
  - A reversible transformation of the genome
- Full-text index in Minute space (FM) index
  - *Paolo Ferragina, and Giovanni Manzini. "Opportunistic data structures with applications." Foundations of Computer Science, 2000. Proceedings. 41st Annual Symposium on. IEEE, 2000.*
  - Implementations: BWA, bowtie and SOAP2
  - How fast? First an intro to “big O” notation

Brief intro to “Big O” notation



The CALCULATED procedure in Figure 3 gives a better, though not optimal, bound. It is conceptually equivalent to the one described in Figure 4, which is simpler to understand. We use the BWT of the reverse (not complemented) reference sequence to test if a substring of W is also a substring of X. Note that to do this test with BWT string B alone would make CALCULATED an $O(|W|^2)$ procedure, rather than $O(|W|)$ as is described in Figure 3.

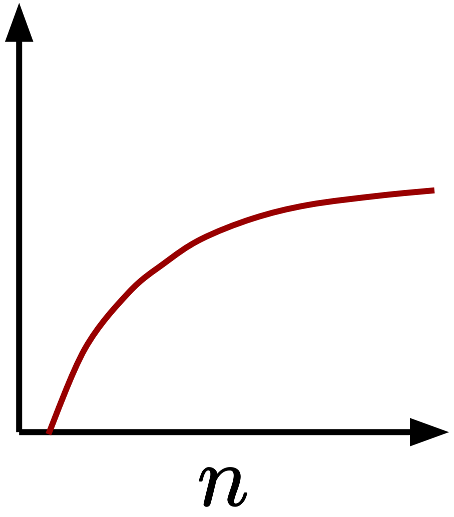
?

from: Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009 Jul 15;25(14):1754-60.

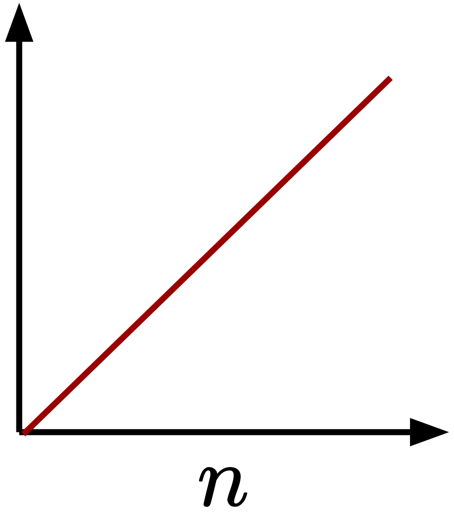
Brief intro to “Big O” notation

- If I have n sequences, how does the amount of time required by the program increase?

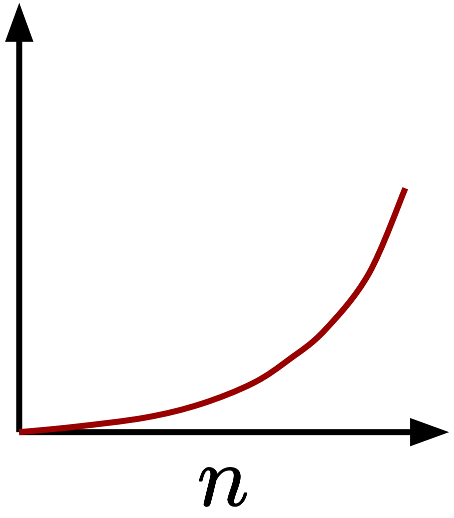
$$log(n)$$



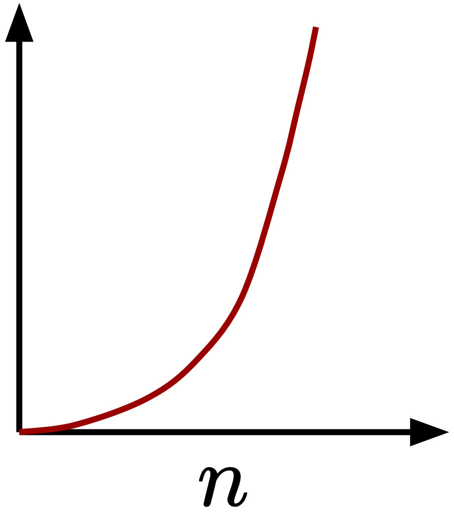
$$n$$



$$n^2$$



$$2^n$$



Brief intro to “Big O” notation

- If I have n sequences, how does the amount of time required by the program increase?

Number of steps

$$3n^2 + 6n + 1$$

$$25n^2 + 5n + 4$$

$$9n^2$$

$$1000n^2$$

$$n^2 - 9$$

$$\longrightarrow O(n^2)$$

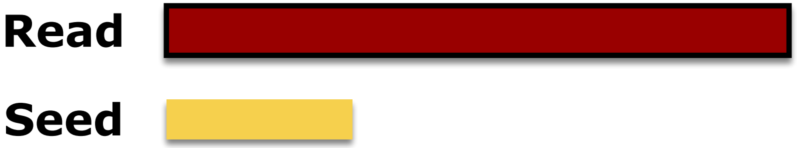
BWT for alignment

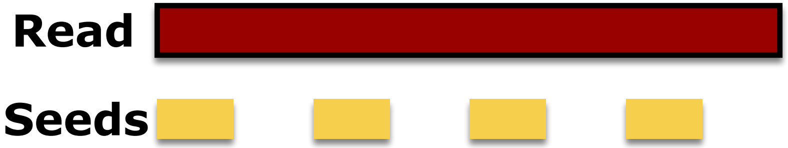
- The FM index uses $O(|L|)$ memory
- Can be searched in $O(|pattern|)$ steps
- Entire FM-index is 1.5Gb for human genome
- FM-index
  - We also need certain other data structures that we did not cover

- Human genome can be effectively indexed and searched using 3Gb RAM!

Implementation in BWA

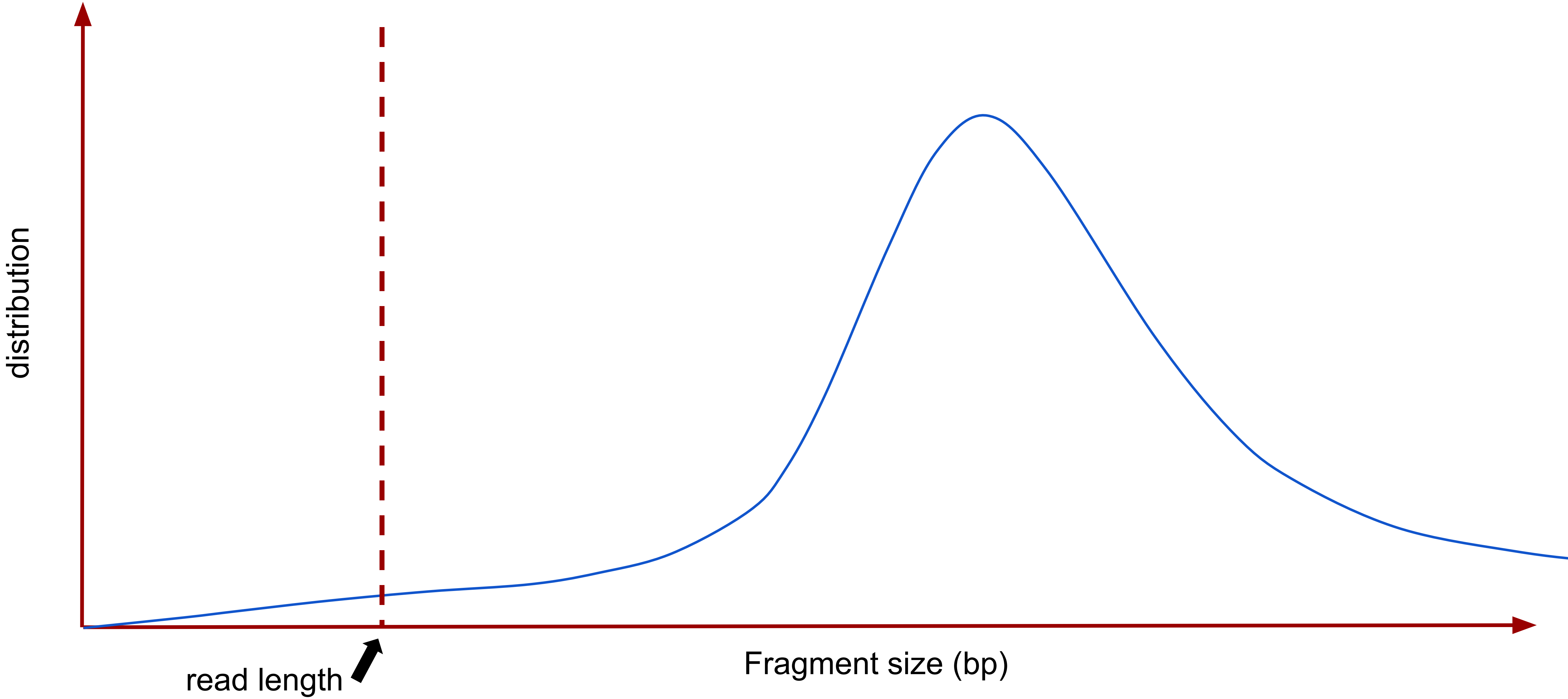
- Burrows Wheeler Aligner (BWA) can use:
  - bwa aln: First ~30nt of read as seed
    - Extend around positions with seed match
    - For short reads
  - bwa mem: Multiple short seeds across the read
    - Extend around positions with several seed matches
    - For longer reads

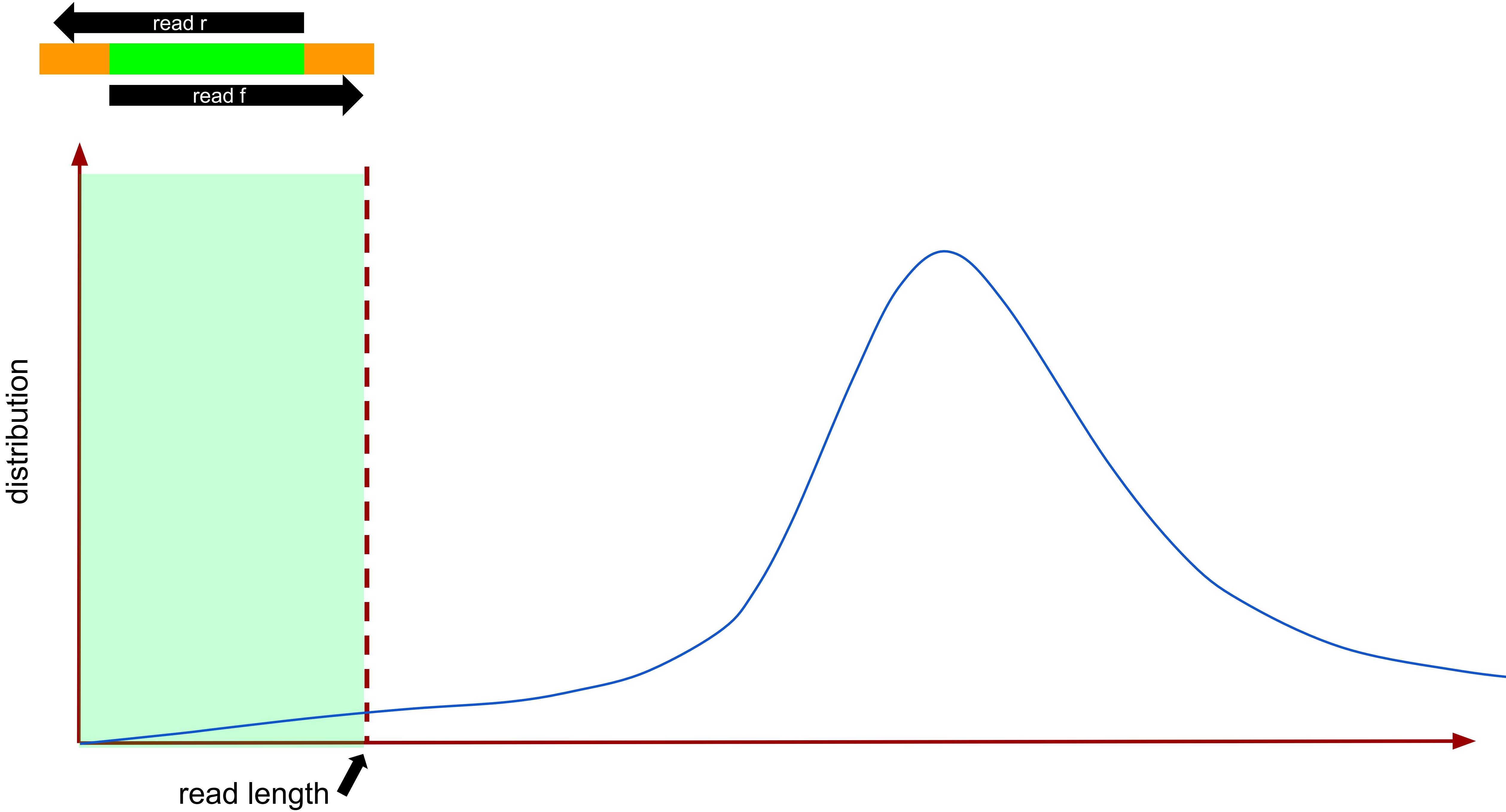


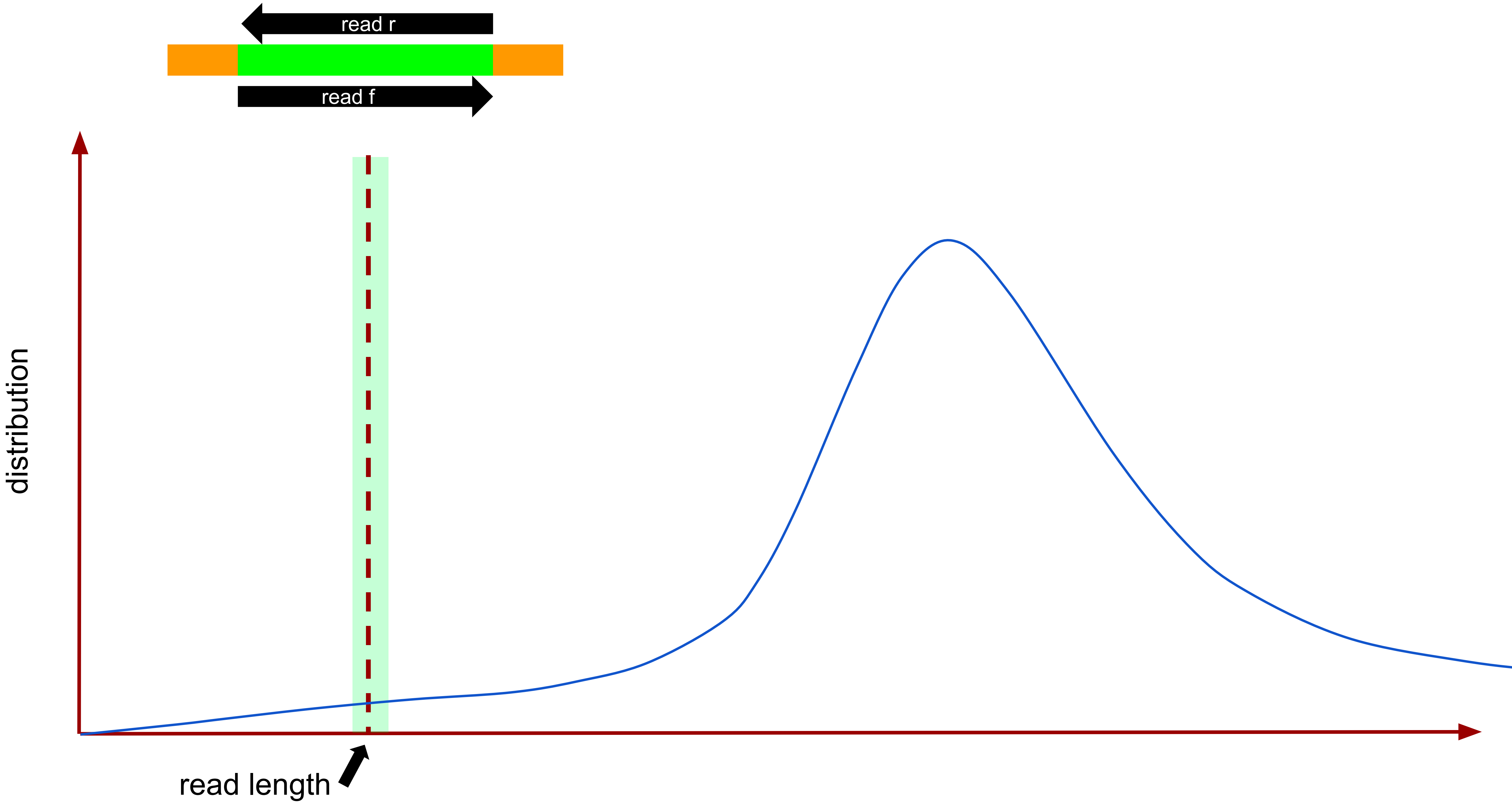


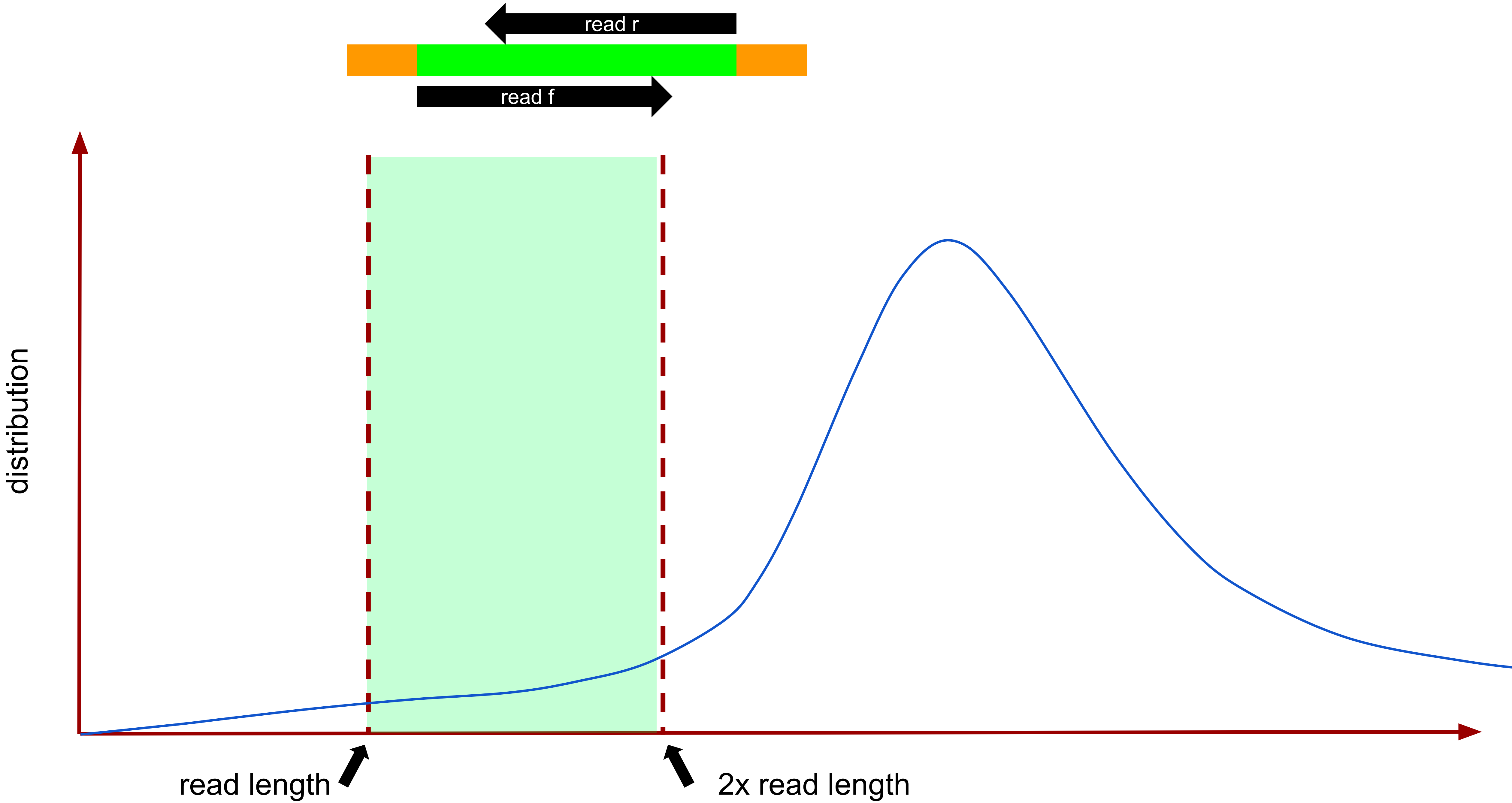
Notes about mapping quality and paired-end

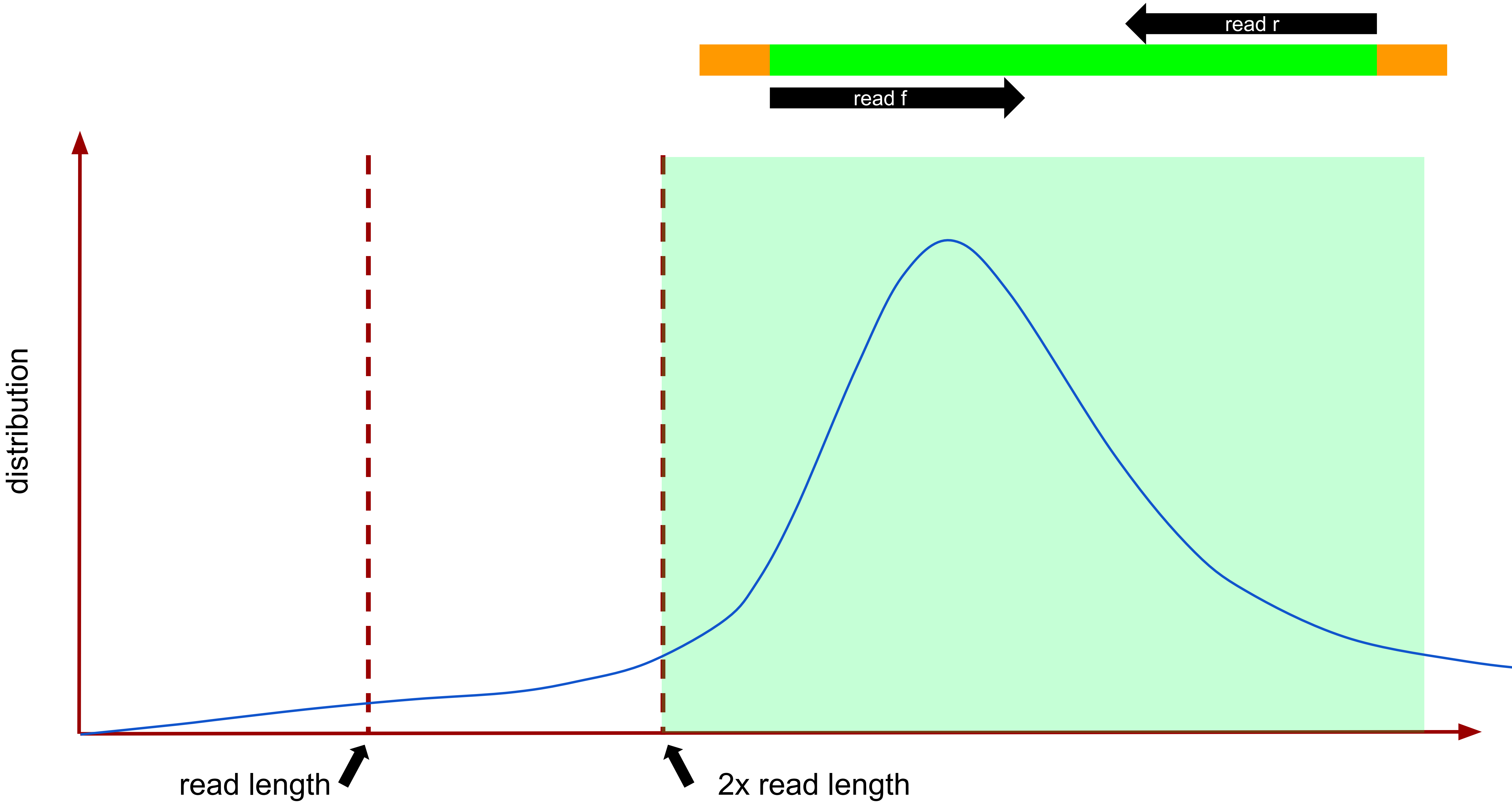
Insert size distribution

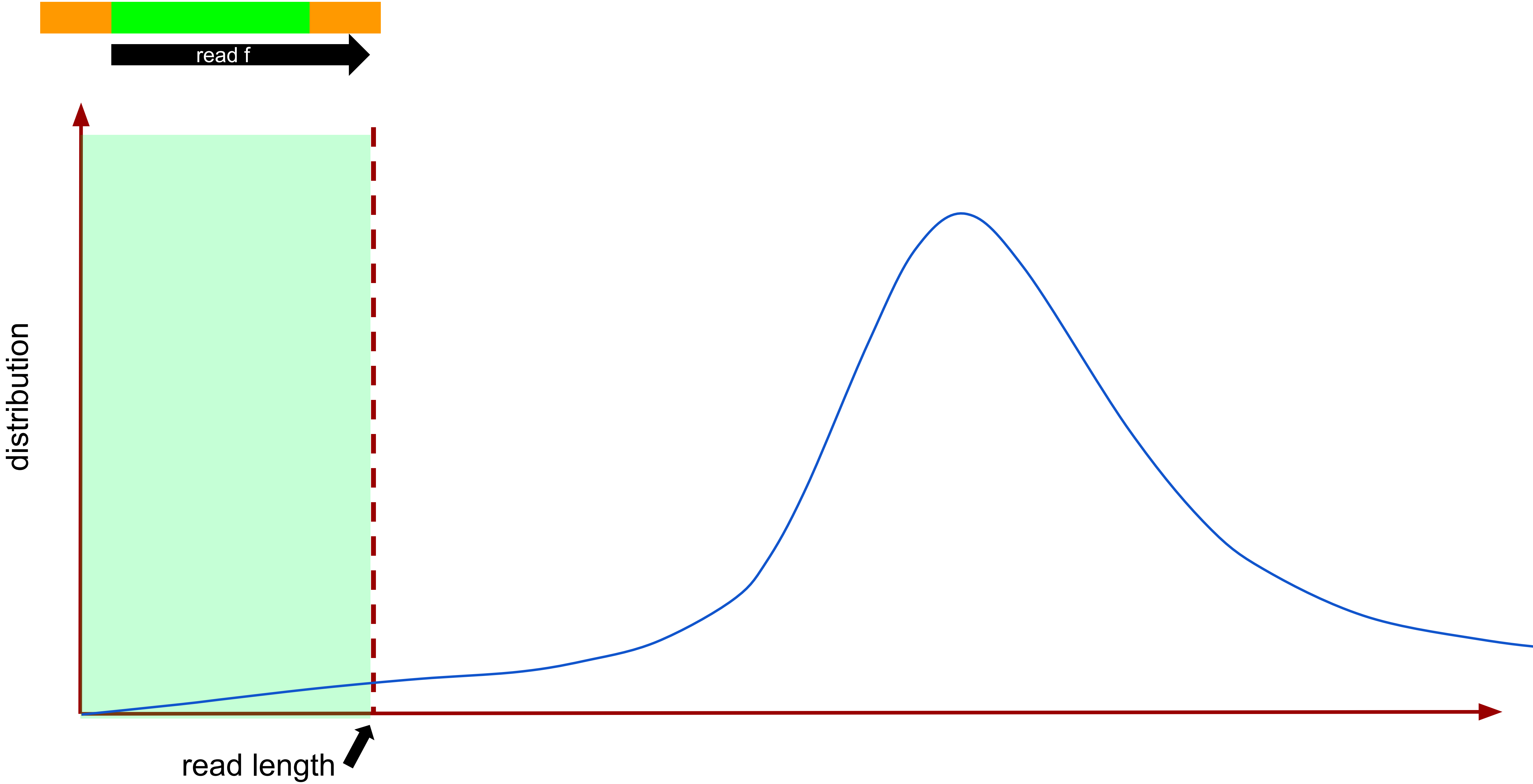


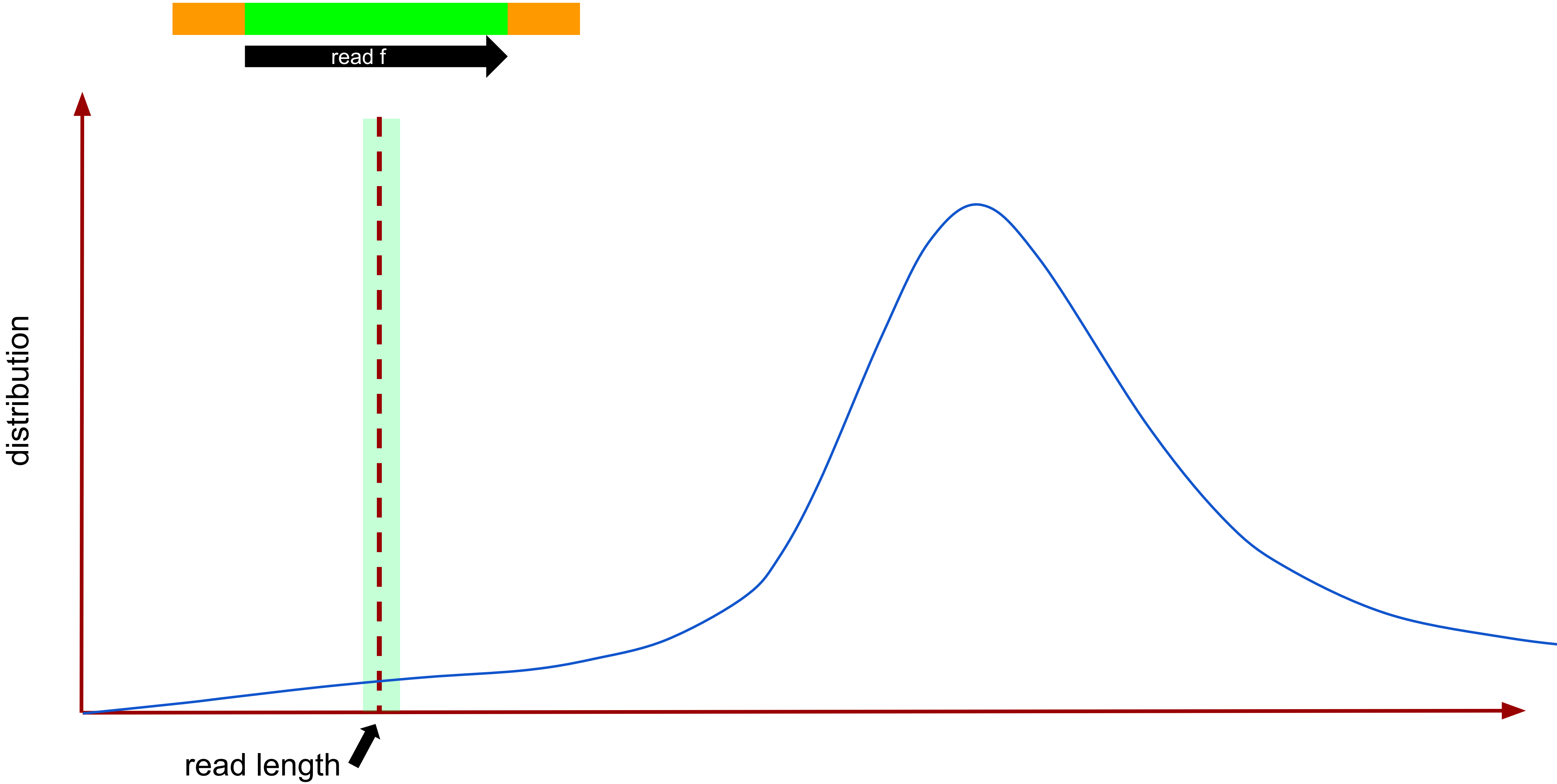

read r
read f
distribution
read length

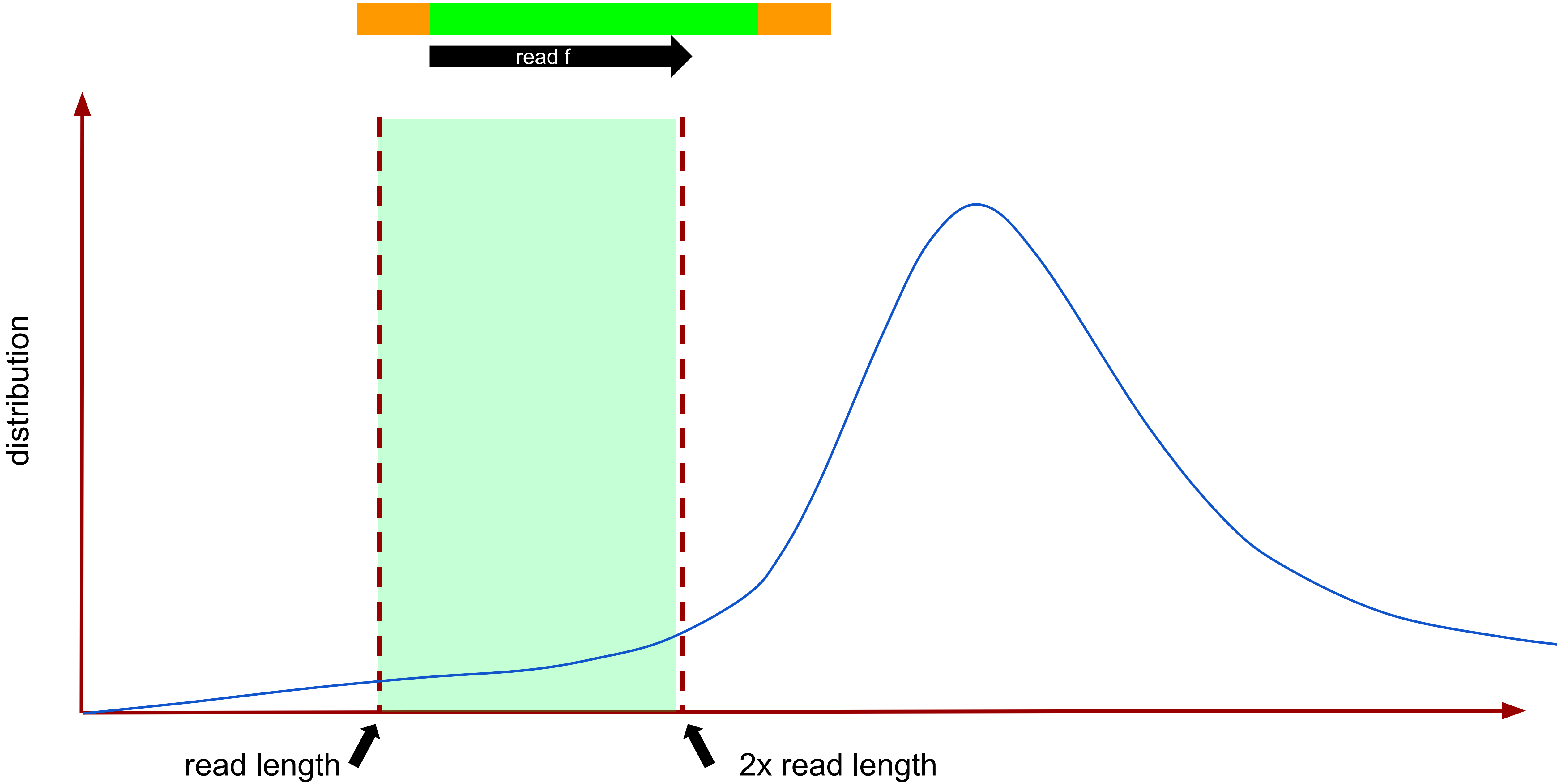

read r
read f
distribution
read length

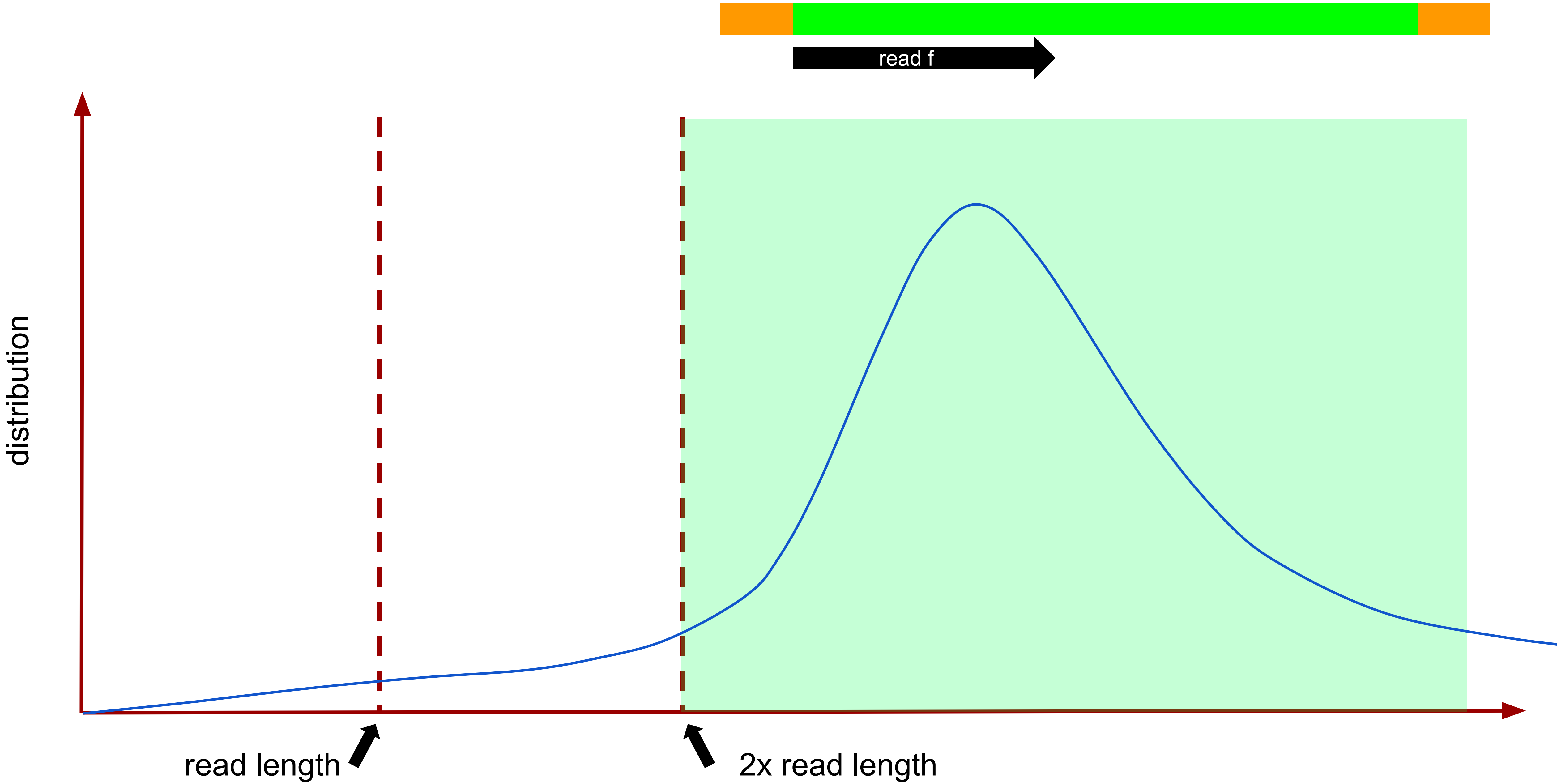

read r
read f
distribution
read length
2x read length


read r
read f
distribution
read length
2x read length


read f
distribution
read length


read f
distribution
read length


read f
distribution
read length
2x read length


read f
distribution
read length
2x read length

Intro to mapping quality

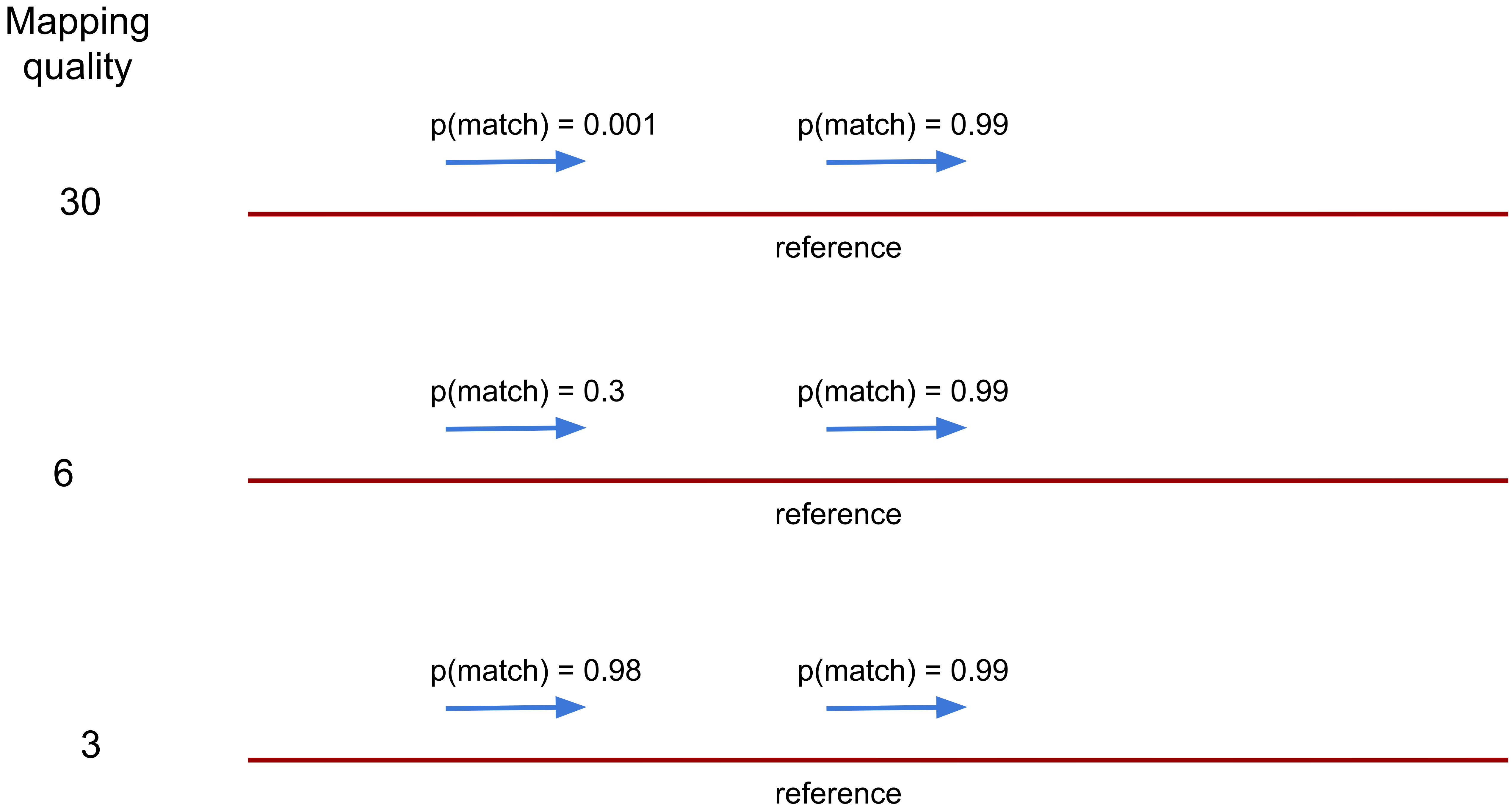
What happens when a sequence has multiple hits to the genome?

Depends on the aligner, Burrows-Wheeler Aligner (BWA) does the following:

- Assign to the genomic location with the best score
- Use other matches to compute the probability of mismapping on a log scale:

$$\text{MAPQ} = -10 \log (\text{P[mismapping]})$$

$$\text{e.g. MAPQ 30} = \text{P[mismapping]} = 1/1000$$



Mapping quality

30

p(match) = 0.001

p(match) = 0.99

reference

6

p(match) = 0.3

p(match) = 0.99

reference

3

p(match) = 0.98

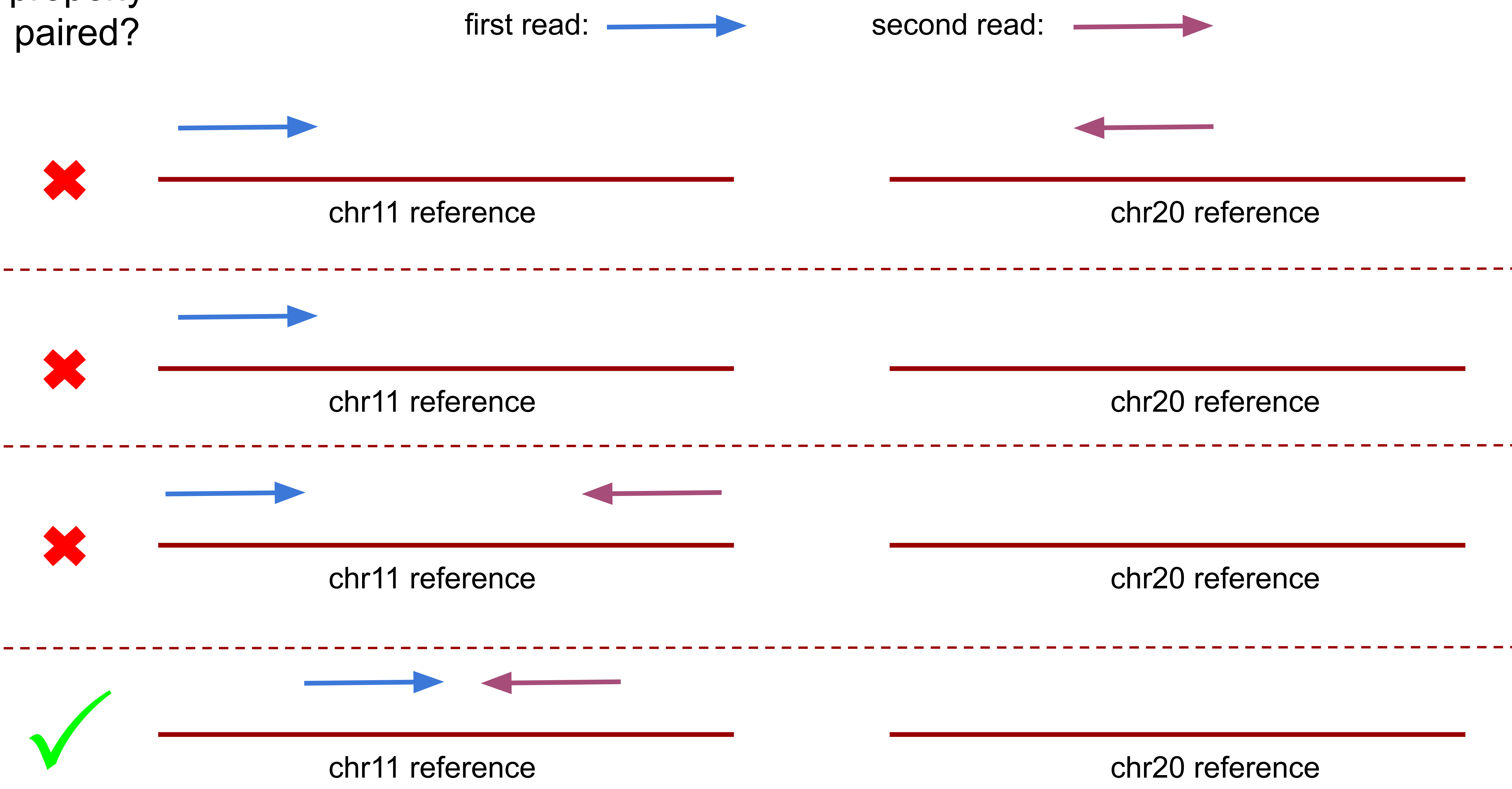
p(match) = 0.99

reference

Intro to “proper pairs” vs “unpaired”

- Some aligners add an extra flag to indicate that 2 paired reads were found:
  - on the same chromosome
  - facing each other (one + strand,   the other - strand)
  - within a “reasonable” distance

properly paired?



first read:

second read:

✗ chr11 reference chr20 reference

✗ chr11 reference chr20 reference

✗ chr11 reference chr20 reference

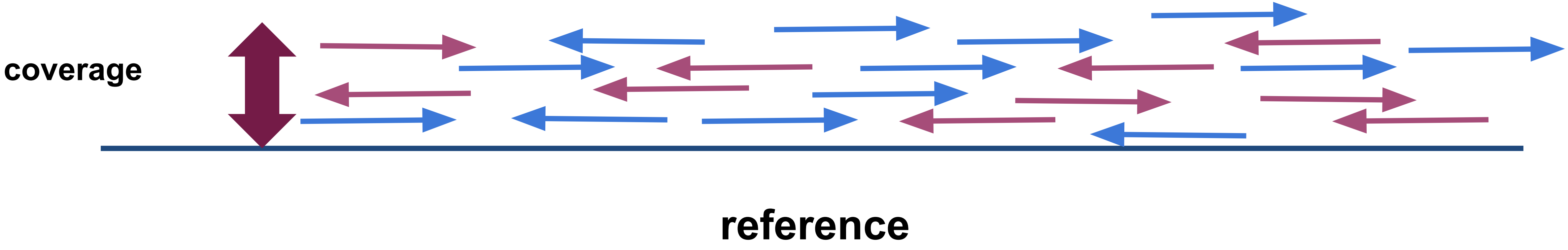
✓ chr11 reference chr20 reference

mapping quality vs mappability

- Mapping quality is often (poorly) approximated for speed
- Use another technique to avoid spurious mappings: genomic mappability
- Mapping quality is per read
- Mappability is for a genomic region

Coverage

- Coverage/depth is how many times that your data covers the genome (on average)



Coverage

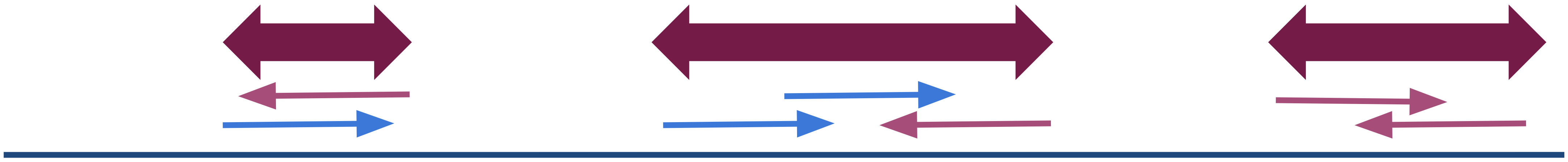
- Coverage/depth is how many times that your data covers the genome (on average)

- Example:
  - N: Number of reads: 5M
  - L: Read length: 100
  - G: Genome size: 5Mbp
  - C = 5M*100/5M = 100X
  - On <u>average</u> there are 100 reads covering each position in the genome

$$C = N \times \frac{L}{G}$$

Breadth of coverage

- breadth of coverage: fraction of the genome covered (1X or more)



reference

SAM/BAM format

- Sequence Alignment / Map format
- BAM = Binary SAM and zipped - always convert to BAM
- Two sections
  - Header: All lines start with “@”
  - Alignments: All other lines

```
@SQ SN:mito_ref  LN:16569
@PG ID:bwa   PN:bwa  VN:0.7.17    CL:bwa samse human_MT.fa test_cut.sai input.fq.gz
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

The header section

```
@SQ  SN:mito_ref  LN:16569
@PG  ID:bwa   PN:bwa   VN:0.7.17    CL:bwa samse human_MT.fa test_cut.sai input.fq.gz
```

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

The alignment section

```
@SQ  SN:mito_ref  LN:16569
@PG  ID:bwa   PN:bwa   VN:0.7.17    CL:bwa samse human_MT.fa test_cut.sai input.fq.gz
```

Contains information like:

- What command line was used to generate this SAM/BAM file?
- What does the reference genomes look like? (chromosome names+length)

```
@SQ  SN:mito_ref  LN:16569
@PG  ID:bwa   PN:bwa   VN:0.7.17    CL:bwa samse human_MT.fa test_cut.sai input.fq.gz
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

Read IDs (should be unique)

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

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

ex:

```
 0 = single-end read, mapped, read mapping to + strand
16 = single-end read, mapped, read mapping to - strand
```

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

name of chromosome (ex: chr1, chr2 ...

```
HN3BX:8:2115:3985:4426 16 mito_re  5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_re  9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_re  7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_re  14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_re  1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

coordinate on chromosome

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

mapping quality

ex:

```
MQ = prob of correct mapping
37 = 99.98%
15 = 96.84%
 0 = 00.00%
```

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M   0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M   0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M   0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M   0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M   0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

CIGAR

Number of operations:
matches (**M**)
insertions (**I**)
deletions (**D**)

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 1 M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0    M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15    M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0    M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0    M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

chromosome of the other pair (not used here)

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

position on the chromosome of the other pair (not used here)

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

length between pairs (not used here)

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

sequence of the reads

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

quality scores of the reads

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

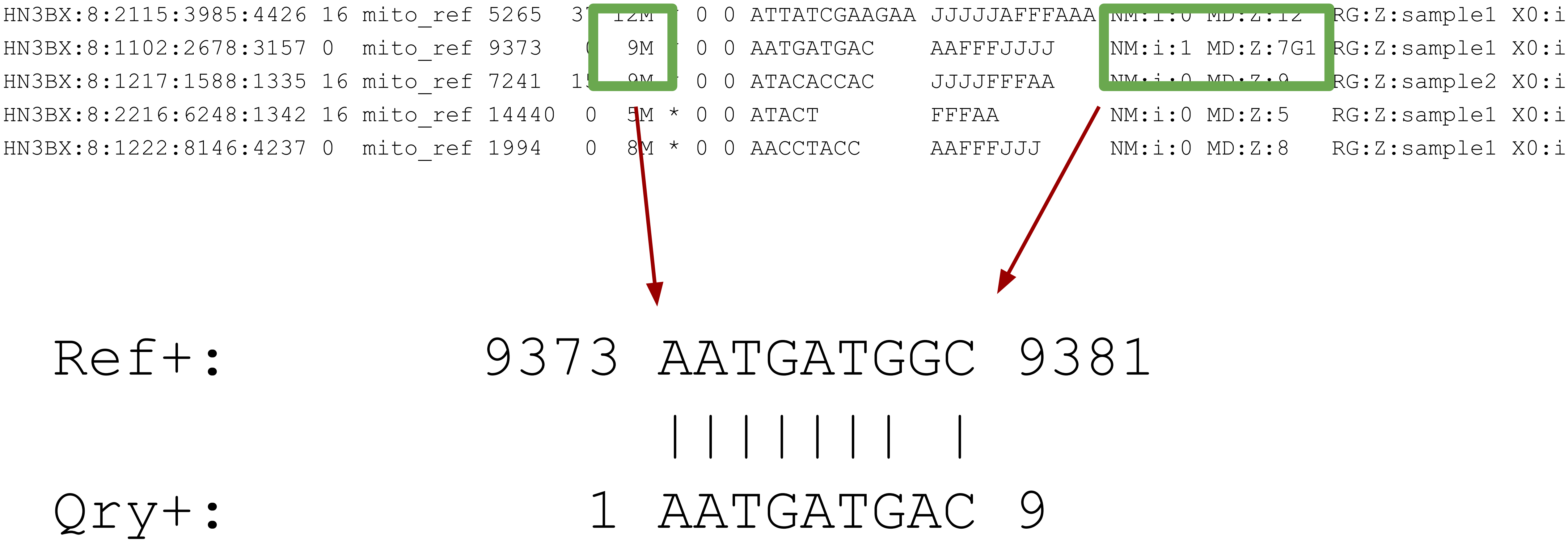
optional flags, tell us:

- how many mismatches in alignment
- total number of matches
- read group

```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

multiplexing: to which read group does the read belong to?

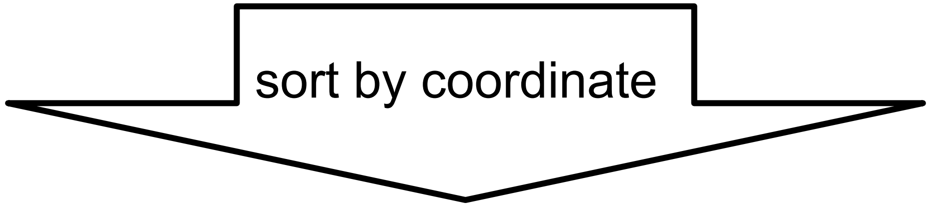
How to reconstruct the alignment? it’s complicated...



```
HN3BX:8:2115:3985:4426 16 mito_ref 5265   37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373    0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241   15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440   0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994    0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

```
Ref+:          9373 AATGATGGC 9381
                    ||||||| |
Qry+:             1 AATGATGAC 9
```

```
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```



sort by coordinate

```
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
```

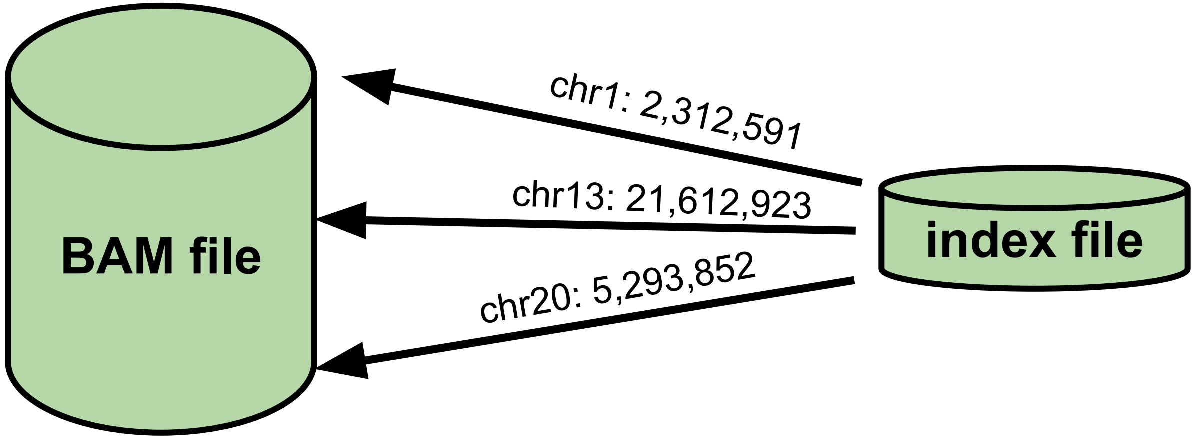
```
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
```

sort by read name

```
HN3BX:8:1102:2678:3157 0  mito_ref 9373   0  9M * 0 0 AATGATGAC    AAFFFJJJJ    NM:i:1 MD:Z:7G1 RG:Z:sample1 X0:i
HN3BX:8:1217:1588:1335 16 mito_ref 7241  15  9M * 0 0 ATACACCAC    JJJJFFFAA    NM:i:0 MD:Z:9   RG:Z:sample2 X0:i
HN3BX:8:1222:8146:4237 0  mito_ref 1994   0  8M * 0 0 AACCTACC     AAFFFJJJ     NM:i:0 MD:Z:8   RG:Z:sample1 X0:i
HN3BX:8:2115:3985:4426 16 mito_ref 5265  37 12M * 0 0 ATTATCGAAGAA JJJJJAFFFAAA NM:i:0 MD:Z:12  RG:Z:sample1 X0:i
HN3BX:8:2216:6248:1342 16 mito_ref 14440  0  5M * 0 0 ATACT        FFFAA        NM:i:0 MD:Z:5   RG:Z:sample1 X0:i
```

BAM indexing

- I want all reads mapping to chrX
- Go through the entire BAM file until we reach chrX
- Better idea: keep a small file telling you where chromosome/coord start:



- Need a BAM file sorted by coordinate
- Is usually a .bai or .csi file

BAM vs CRAM

- Idea: why store the original reads? You only the differences to a reference
- Can be 30-60% smaller
- Good for long term storage
- BAM can be converted to CRAM
- Can be indexed

Exercise time!

http://teaching.healthtech.dtu.dk/22126/index.php/Alignment_exercise