# Analysis of Next Generation Sequencing data: Read Mapping

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December 3, 2014

# Center for Integrative Bioinformatics Vienna

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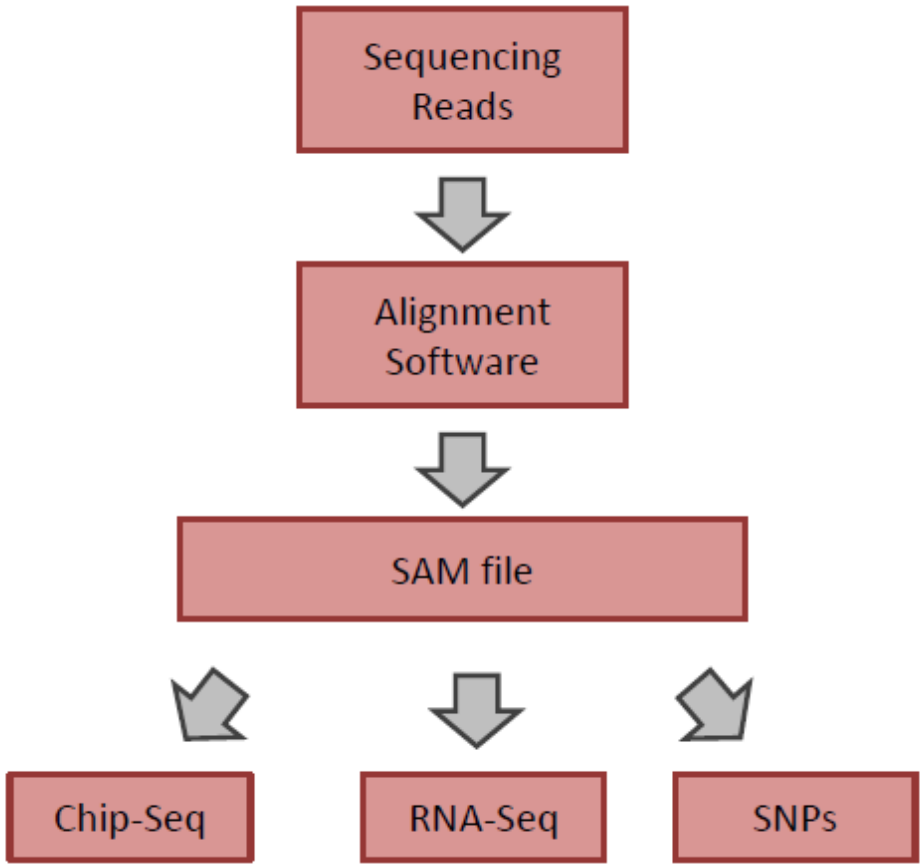
# Next generation sequencing data analysis



# Overview

1. **General:**
   1. What is read mapping?
   2. Why do we need it?
   3. Sequence alignments (optimal)
   4. Seed and extend (heuristic)
   5. Tree based (heuristic)
2. **Seed and extend:**
   1. How does it work?
   2. Hash-table
   3. Sequence alignment optimizations
   4. Applications

# Making sense of NGS data

DNA copies of the genome

Sequence Reads

The Call–Chronicle–Examiner

SAN FRANCISCO, THURSDAY, APRIL 19, 1906.

EARTHQUAKE AND FIRE: SAN FRANCISCO IN RUINS

NO HOPE LEFT FOR SAFETY OF ANY BUILDINGS

BLOW BUILDINGS UP TO CHECK FLAMES

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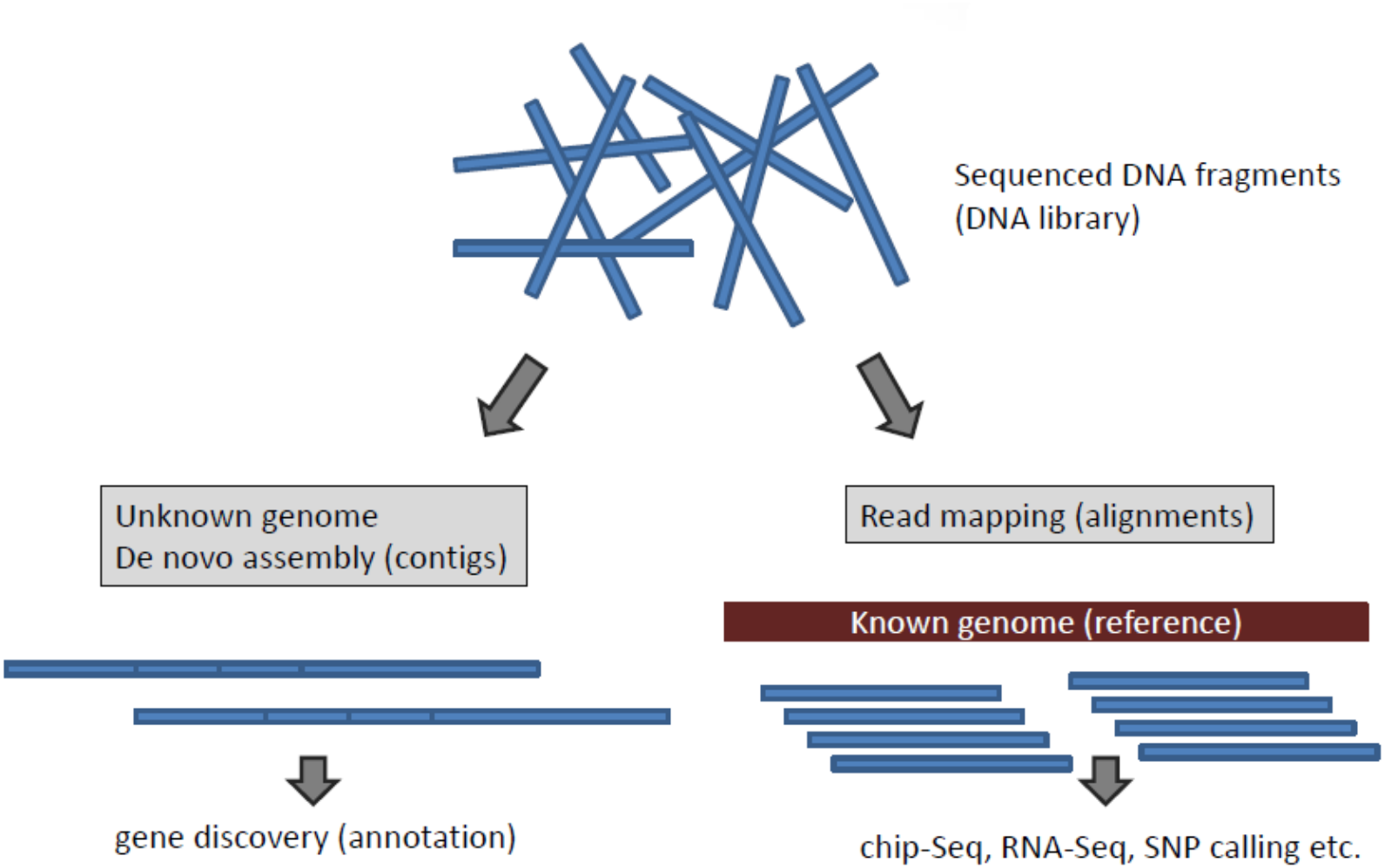

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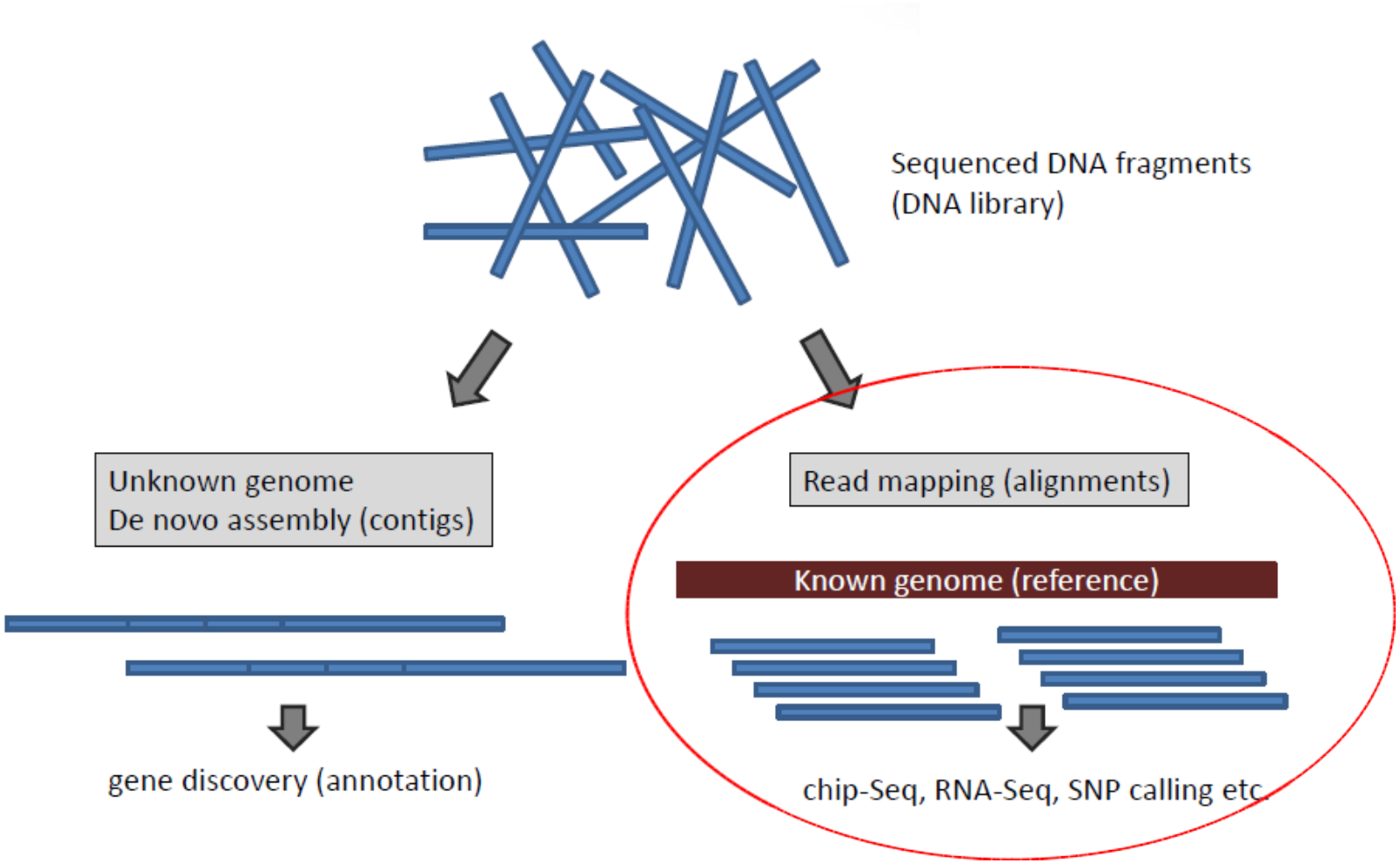

# Input data

- FASTQ file:

```
$ head SRR094861.fastq
@SRR094861.1 HWI-EAS206_23_30H0YAAXX:5:1:709:724 length=36
ACCTCCCGCCCCCNACNGCNCCCCCGTGCACCTCCC
+SRR094861.1 HWI-EAS206_23_30H0YAAXX:5:1:709:724 length=36
555555555555!%5!55!555555$555(.$2.2
@SRR094861.2 HWI-EAS206_23_30H0YAAXX:5:1:1460:1251 length=36
AAACAAGCTAACATGACTAACACCCTTAATTCCATC
+SRR094861.2 HWI-EAS206_23_30H0YAAXX:5:1:1460:1251 length=36
555555555555555555555555555555121-11
@SRR094861.3 HWI-EAS206_23_30H0YAAXX:5:1:899:1936 length=36
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```

1. sequence id
2. sequence content
3. + (placeholder)
4. quality string

# Read mapping (1)



- Finding the region of the reference genome that shows the highest similarity to a given read

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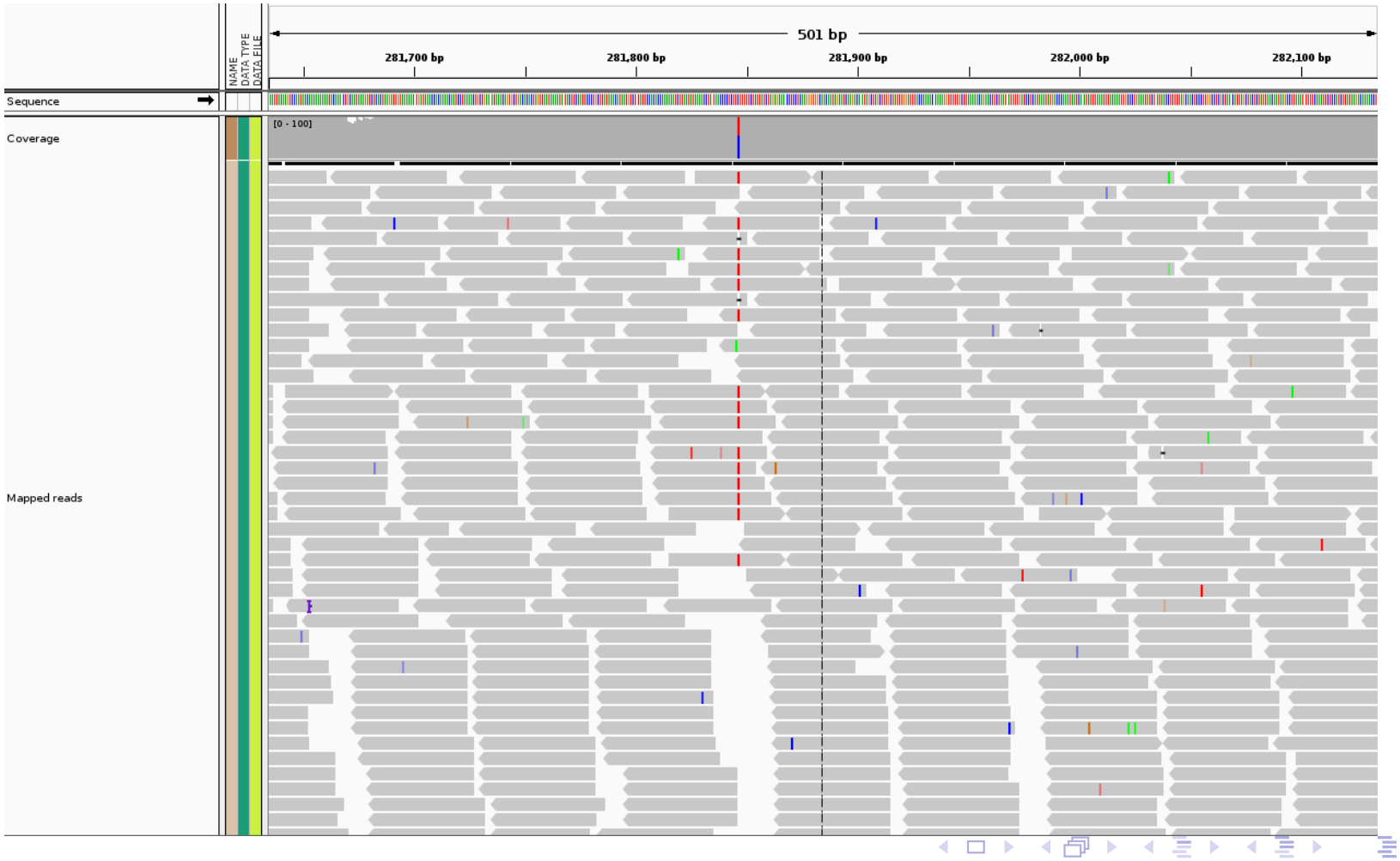
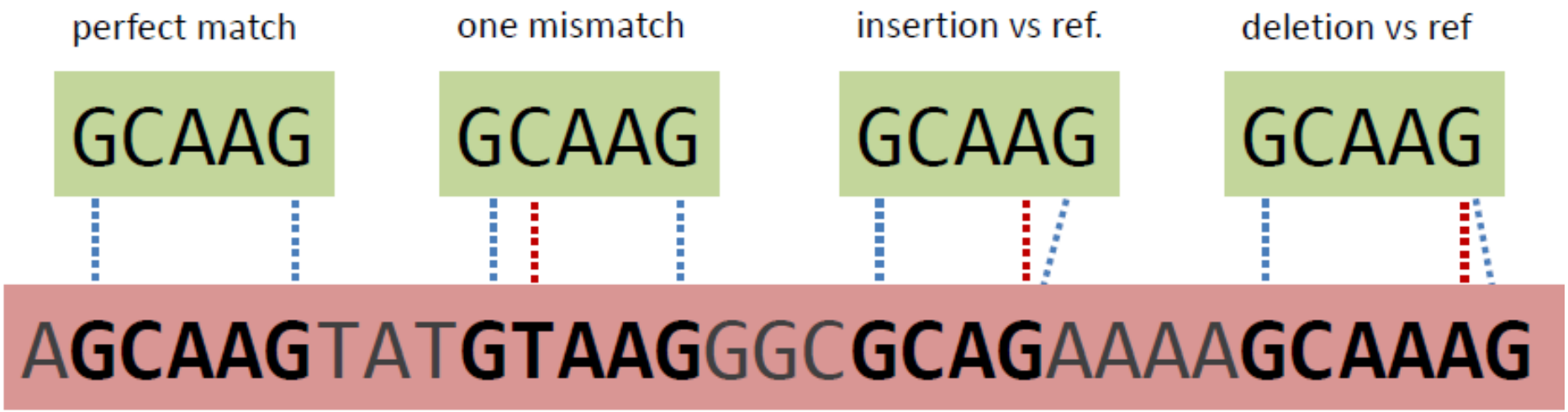


# Read mapping (2)





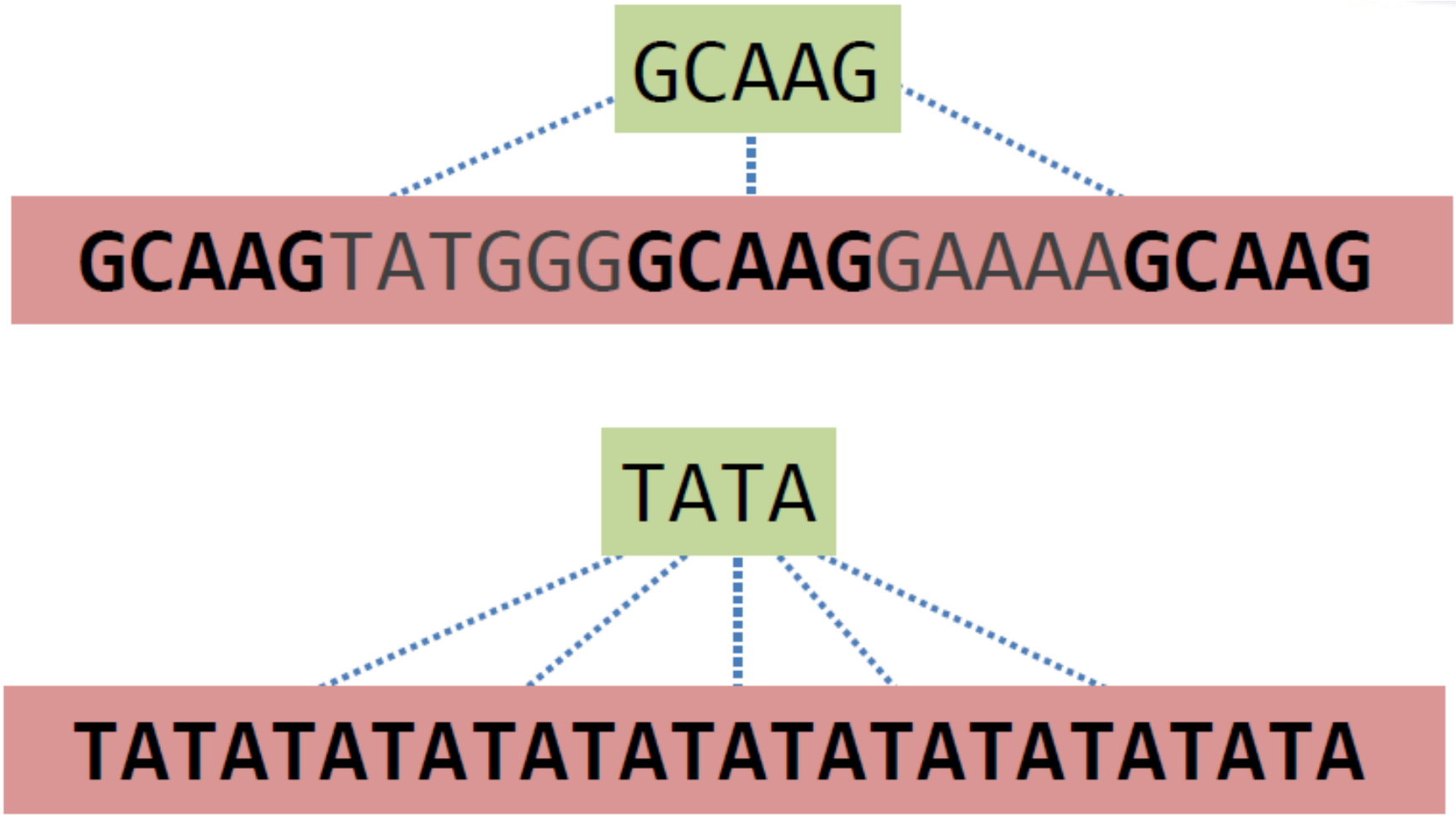

**NOTE: mismatches or indels can be longer than 1 base!**

**It gets complicated very quickly**

Alignment scoring depends on mismatch scoring (different across bases!), gap open, gap extension penalties

# Read mapping (3)





# Difficulties



- Size of reference genome (human: 3 GB, some plant genomes even bigger)
- Number of reads ($> 100,000,000$)
- Differences between read and reference sequence. How to weight them.
- Repeats! Some parts of the genome are unique, some are repeated thousands of times

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- Arranging two or more sequences such as to maximize the length of the common regions between the two
- Operations: insert gap into sequence 1, insert gap into sequence 2, accept mismatch
- Well developed field of research

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Given two sequences $A$ and $B$ and a scoring function for two characters $a$ and $b$

$$S(a,b) = \begin{cases} +5 & \text{if } a = b \text{ (match)} \\ -2 & \text{if } a \neq b \text{ (mismatch)} \\ -6 & \text{if } a \text{ or } b \text{ indel (gap)} \end{cases}$$

to score each alignment column. Then we are looking for that alignment, that gives us the highest score $S(A,B)$.

For example:

**T G C T C G T A**

**T – – T C A T A**

+5−6−6+5+5−2+5+5 =11

# Global Alignment: Needleman-Wunsch

Given sequences $A$ and $B$ and scoring function $s(a,b) = \begin{cases} +5 & a = b \\ -2 & a \neq b \\ -6 & a \text{ or } b \text{ indel} \end{cases}$

Resulting alignment and score:

- Initialize an $N \times M$ matrix with the sequences $A$ and $B$ of length $M$ and $N$.
- Starting at the upper left corner set the intermediate scoring value $\sigma(i,j) = \max \begin{cases} \sigma(i-1,j-1) + s(A_i, B_j) & \text{match/mismatch} \\ \sigma(i-1,j) + s(A_i, -) & \text{gap in } B \\ \sigma(i,j-1) + s(B_i, -) & \text{gap in } A \end{cases}$
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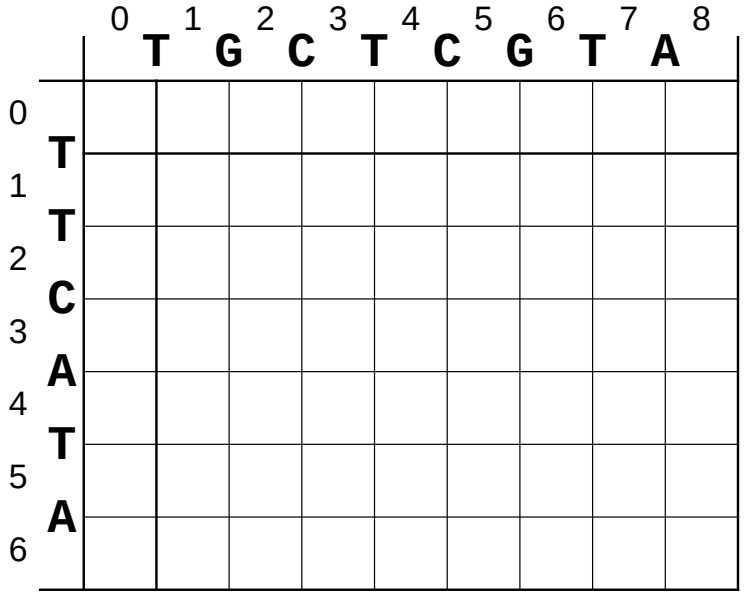


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| | | 0 | 1 T | 2 G | 3 C | 4 T | 5 C | 6 G | 7 T | 8 A |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | 0 | −6 | −12 | −18 | −24 | −30 | −36 | −42 | −48 |
| 1 | T | −6 | | | | | | | | |
| 2 | T | −12 | | | | | | | | |
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|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | 0 | −6 | −12 | −18 | −24 | −30 | −36 | −42 | −48 |
| 1 | T | −6 | 5 | | | | | | | |
| 2 | T | −12 | | | | | | | | |
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| 1 | T | −6 | 5 | −1 | | | | | | |
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**T G C T C G T A**
**T − − T C A T A**
+5−6−6+5+5−2+5+5 =11

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| | | 0 T | 1 G | 2 C | 3 T | 4 C | 5 G | 6 T | 7 A | 8 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | 0 | −6 | −12 | −18 | −24 | −30 | −36 | −42 | −48 |
| 1 | T | −6 | 5 | −1 | −7 | −13 | −19 | −25 | −31 | −37 |
| 2 | T | −12 | −1 | 3 | −3 | −2 | −8 | −14 | −20 | −26 |
| 3 | C | −18 | −7 | −3 | 8 | 2 | 3 | −3 | −9 | −15 |
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Resulting alignment and score:

**T G C T C G T A**
**T − − T C A T A**
+5−6−6+5+5−2+5+5 =11

- Initialize an $N \times M$ matrix with the sequences $A$ and $B$ of length $M$ and $N$.
- Starting at the upper left corner set the intermediate scoring value $\sigma(i,j) =$
$$\max \begin{cases} \sigma(i-1,j-1) + s(A_i, B_j) & \text{match/mismatch} \\ \sigma(i-1,j) + s(A_i, -) & \text{gap in } B \\ \sigma(i,j-1) + s(B_i, -) & \text{gap in } A \end{cases}$$
- Optimal score for can be found at $\sigma(N,M)$.
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# Local alignment (Smith-Waterman algorithm)



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Seq1: GTATACGATTCTTATCTTCTCGTTGTGGTTTCTTA

Seq2: AAAAAAAAAAAAATTCTGTGTTGAAAAAAAAA
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- Instead of looking at the total sequence, the Smith-Waterman algorithm compares segments of all possible lengths and optimizes the similarity measure
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# Heuristic approaches



- Heuristic refers to experience-based techniques for problem solving that find a solution which is not guaranteed to be optimal, but good enough for a given set of goals
  - Seed and extend (BLAST)
  - Tree/Trie based approach (suffix/prefix trees, burrows wheeler transformation, etc.)

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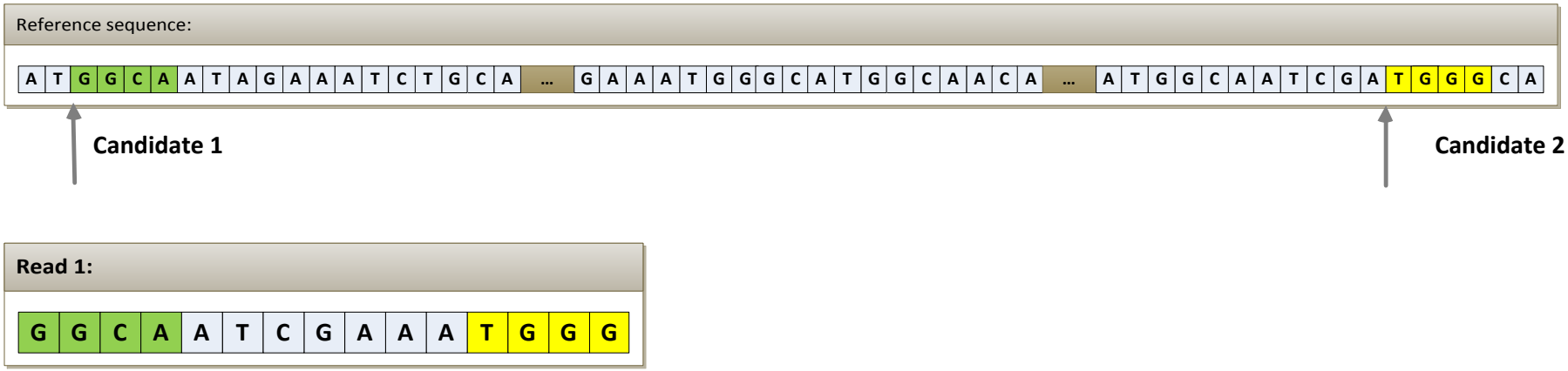
1. Candidate mapping region search: identification of perfectly matching sub regions using an index data structure
2. Computation of Smith-Waterman alignment scores for candidates
3. Computation of full alignment only for the highest scoring candidate



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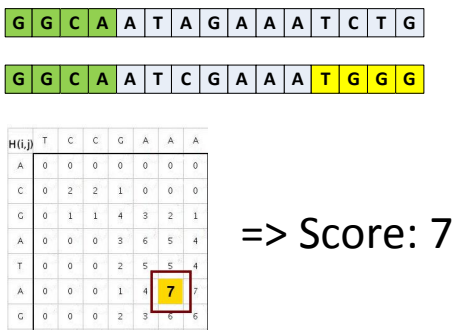
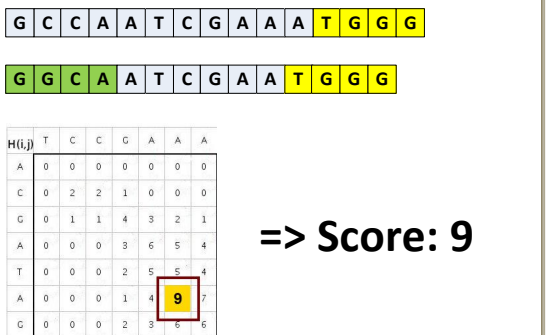



=> Score: 7

**Candidate 2**




**=> Score: 9**

...

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| H(i,j) | T | C | C | G | A | A | A |
|---|---|---|---|---|---|---|---|
| G | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | 0 | 0 | 0 | 0 | 2 | 2 | 2 |
| T | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| C | 0 | 2 | 2 | 1 | 0 | 0 | 0 |
| C | 0 | 2 | 4 | 3 | 2 | 1 | 0 |
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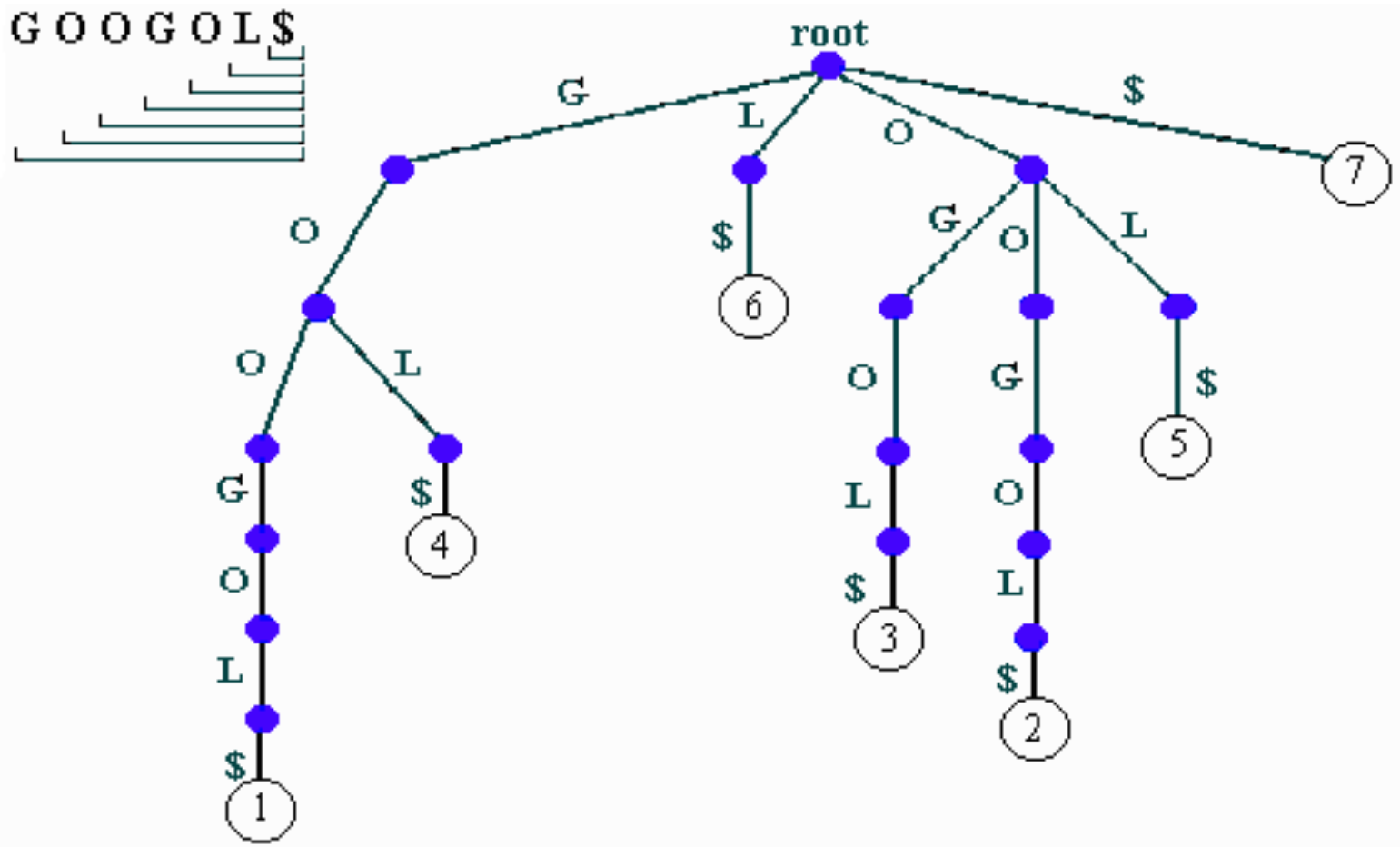
| A | 0 | 0 | 1 | 1 | 4 | 7 | 9 |
| T | 0 | 0 | 0 | 0 | 3 | 6 | 8 |
| G | 0 | 2 | 2 | 1 | 2 | 5 | 7 |

**Candidate 2**

| G | C | C | A | A | T | C | G | A | A | A | T | G | G | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | G | C | A | A | T | C | G | A | A | - | T | G | G | G |

# Suffix tree

- Search “GOL” in “GOOGOL”



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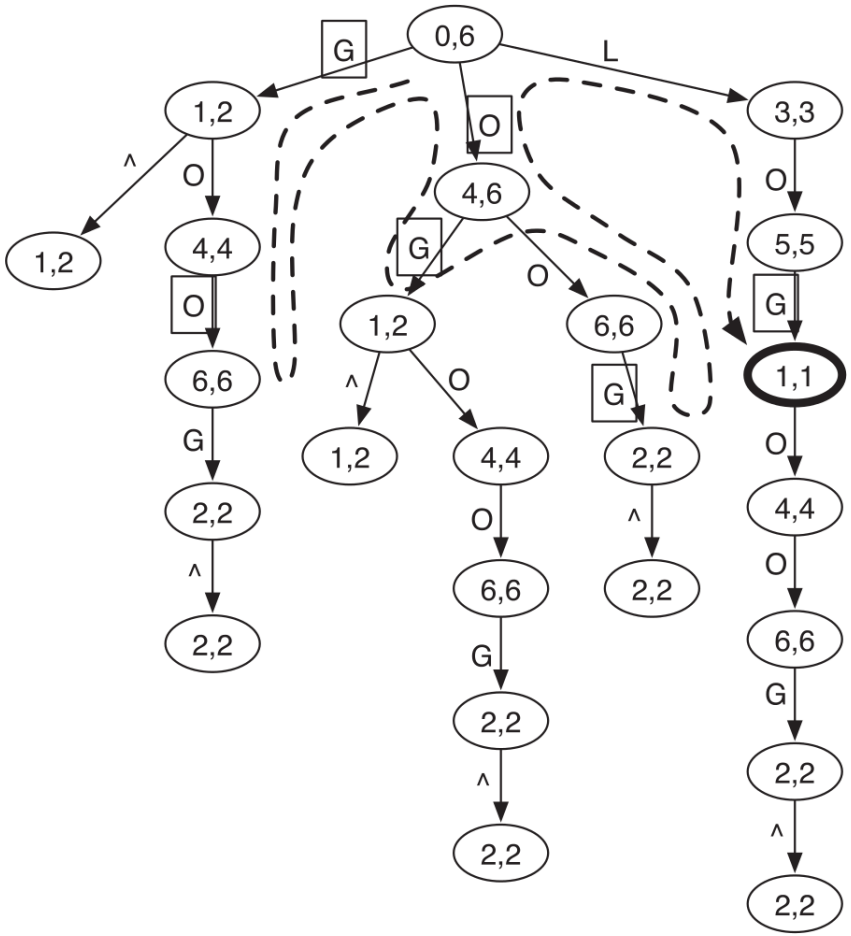
- Search “LOL” in “GOOGOL”



# Seed and extend vs. Trees



Seed and extend:

- High memory usage
- Typically slow(er) due to time consuming alignment computation
- High sensitivity
- Can handle a high number of differences between the read and the references

Trees:

- Fast
- Memory efficient
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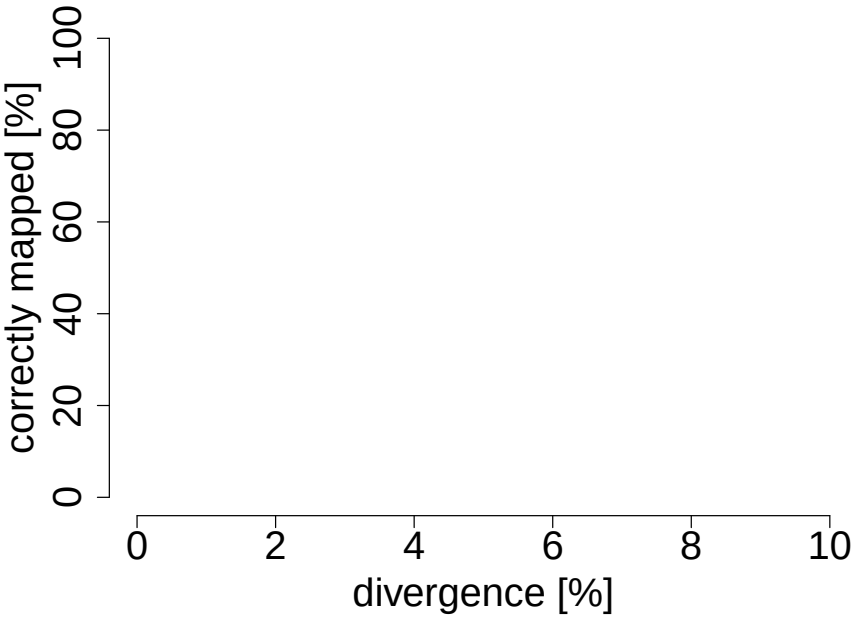
# Read mapper comparison (1)



- Reference genome: *A. thaliana*
- Genomes size: $\sim$ 110 MB
- We simulated 11 data sets with 5 million, 100 bp reads
- 0 to 10 % difference between sequenced and reference genome
- 2% simulated sequencing error
- Programs: BWA(-SW), Bowtie2, Stampy
- Comparison of runtime and number of correctly mapped reads

# Read mapper comparison (2)





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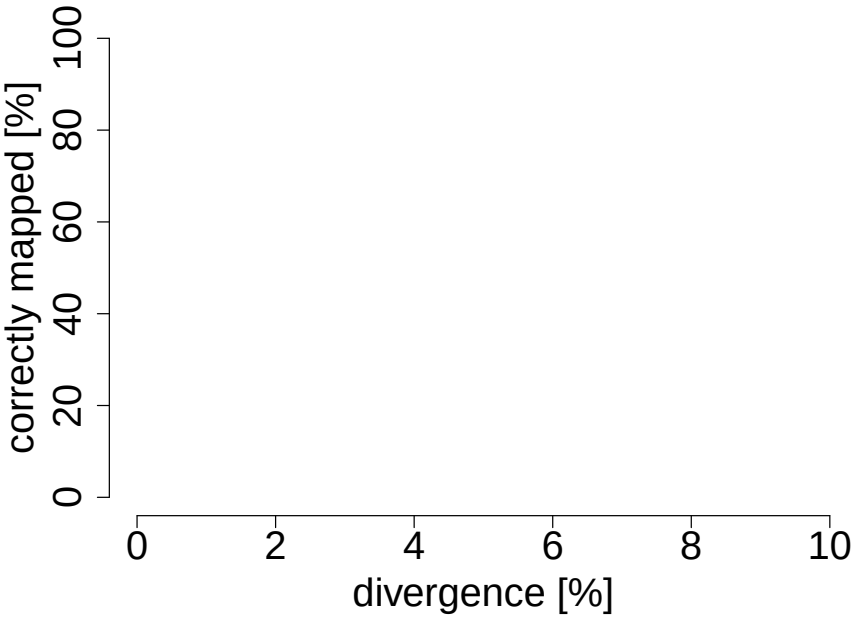


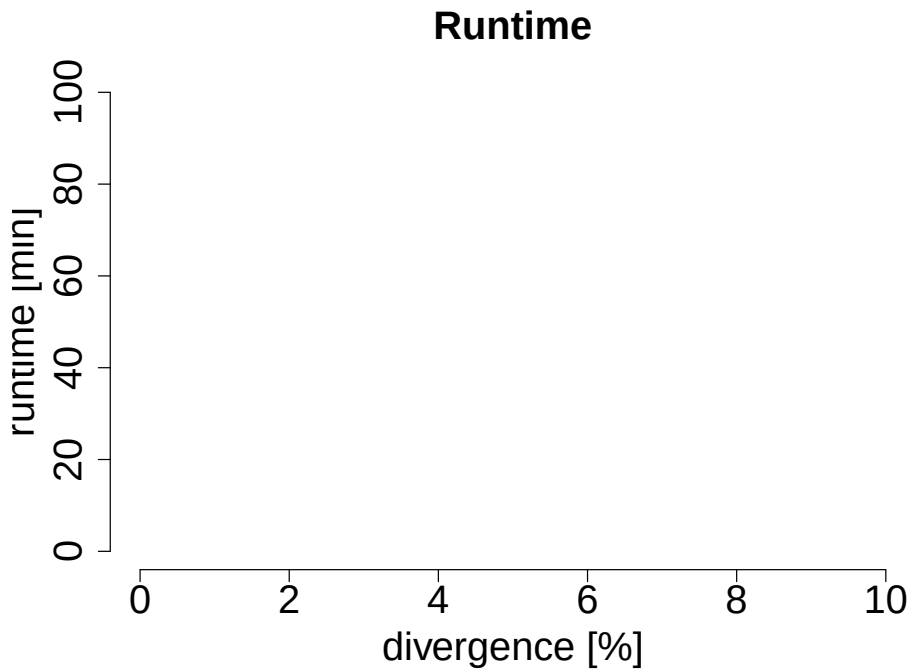

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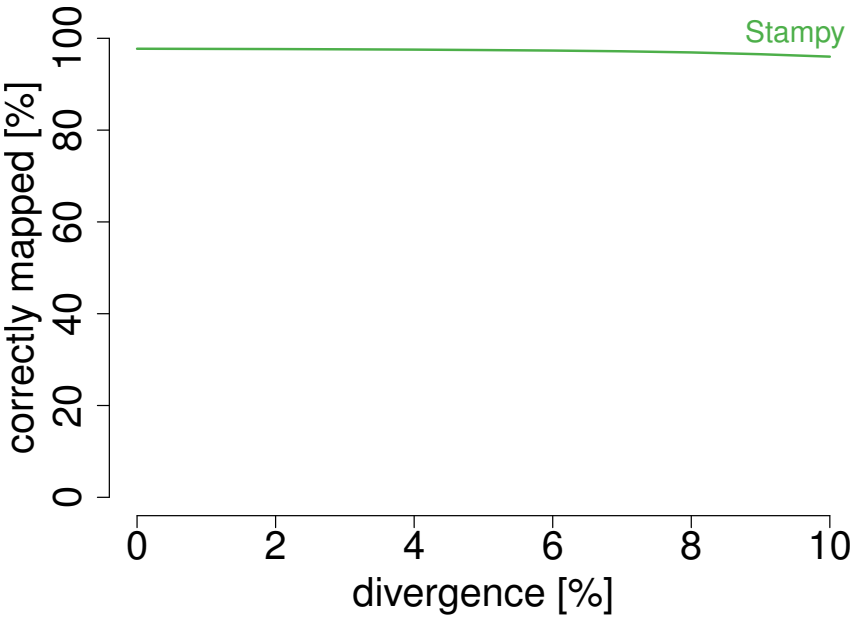


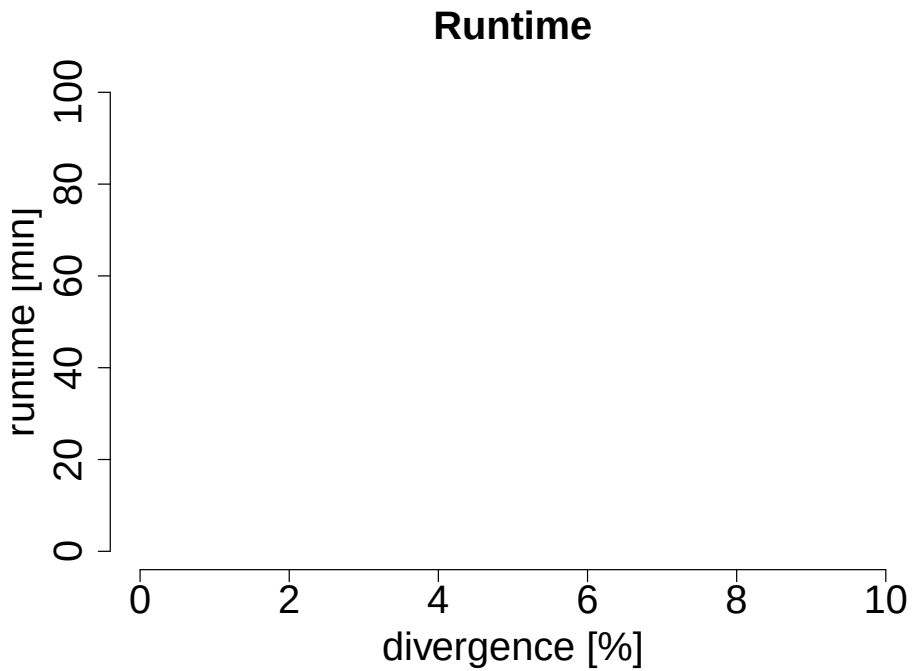

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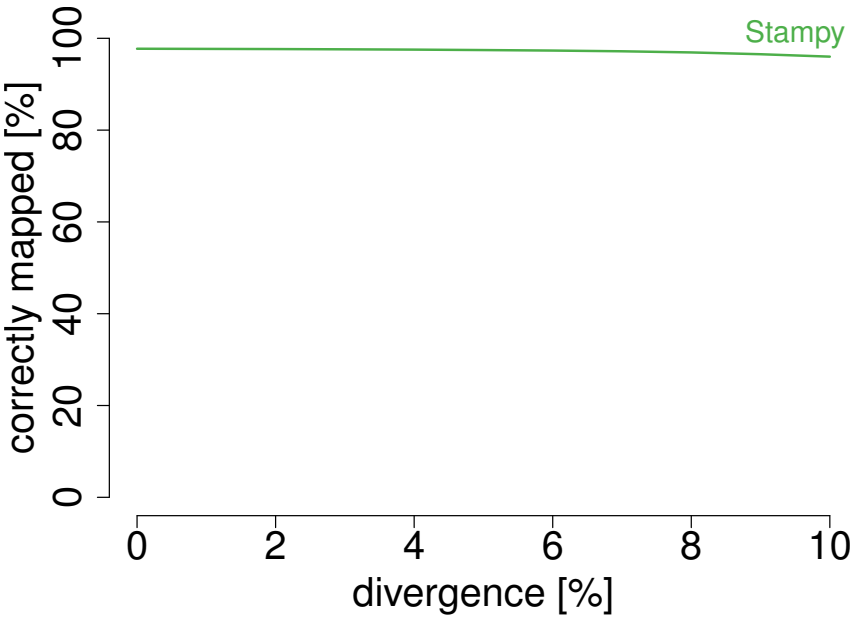


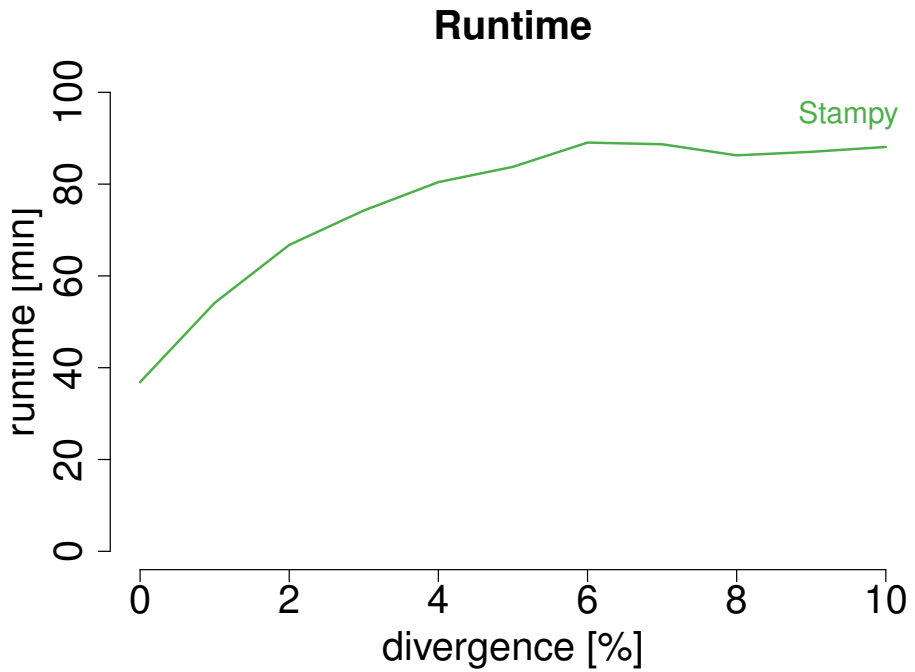

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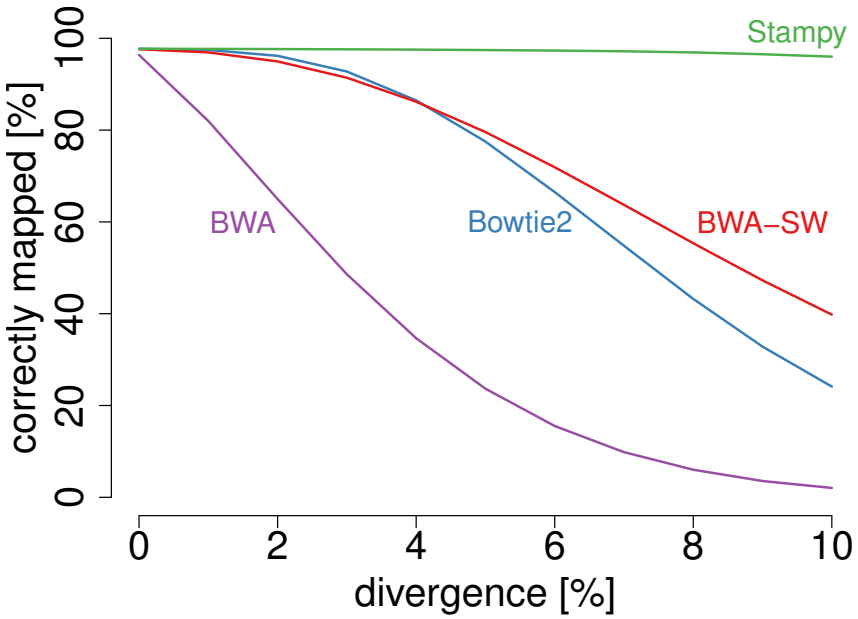


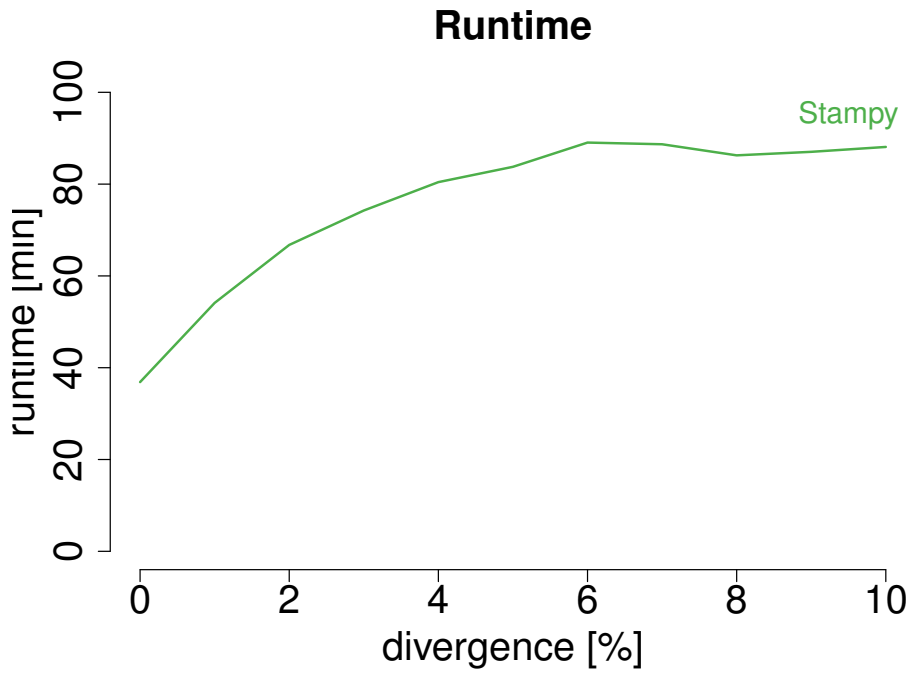

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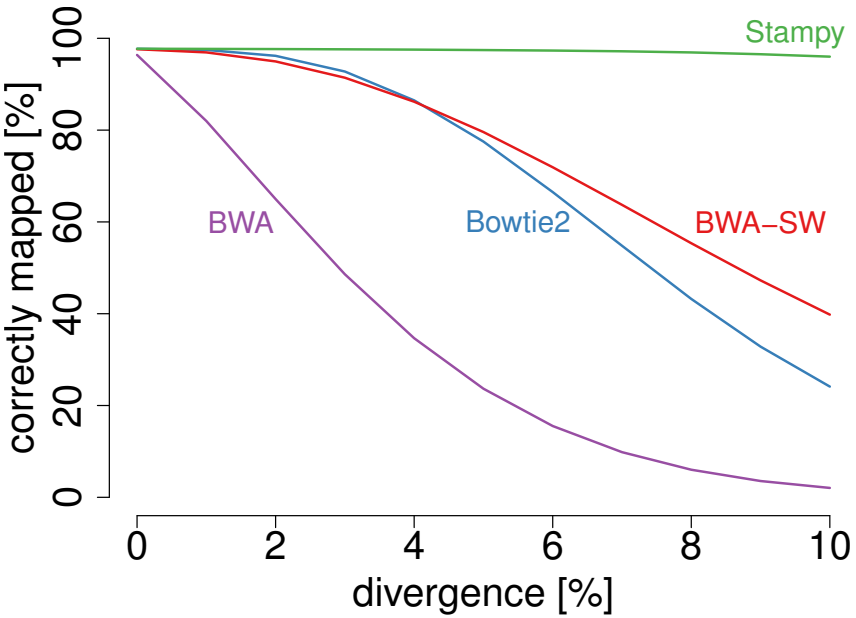


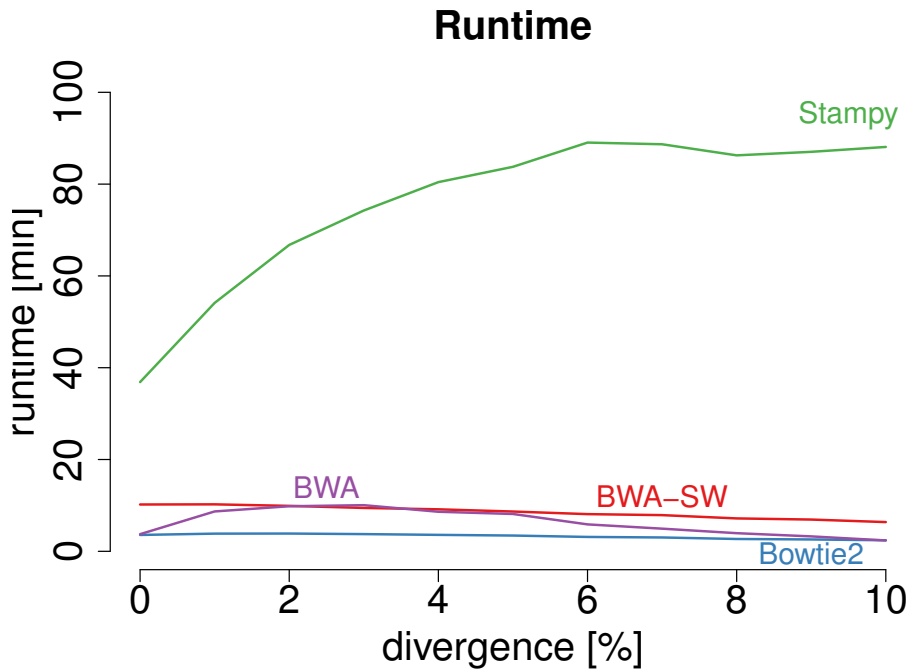

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- Uses sequence alignments to get high mapping sensitivity
- Goal: as fast or faster then tree based approaches
- Optimizations:
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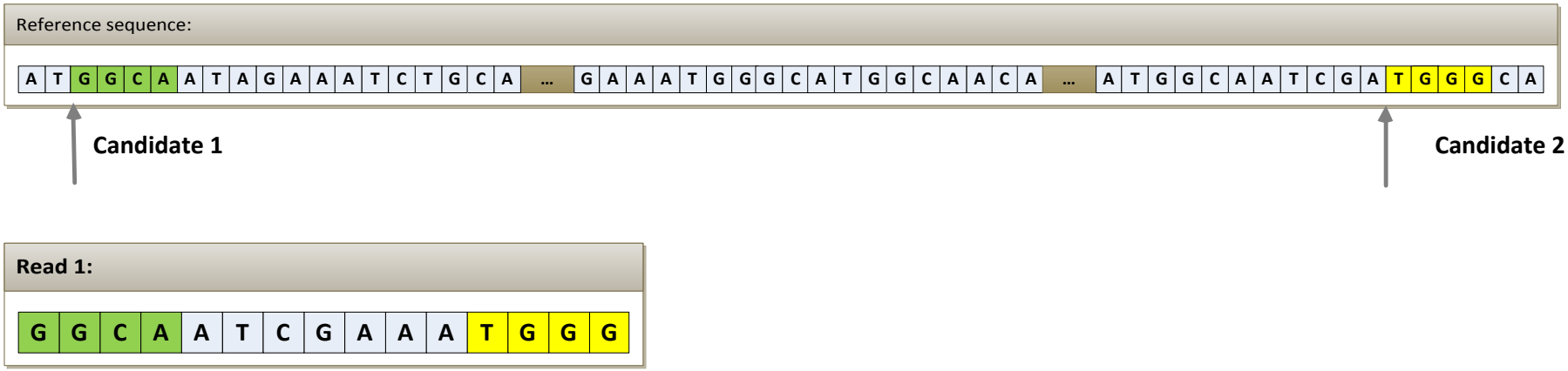

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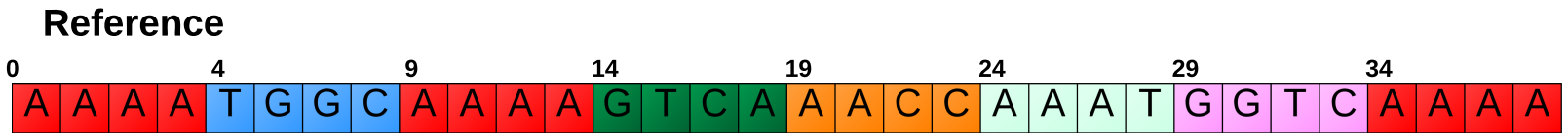


**Objective:**

Fast identification of genomic positions given a k-mer

# Hash-table



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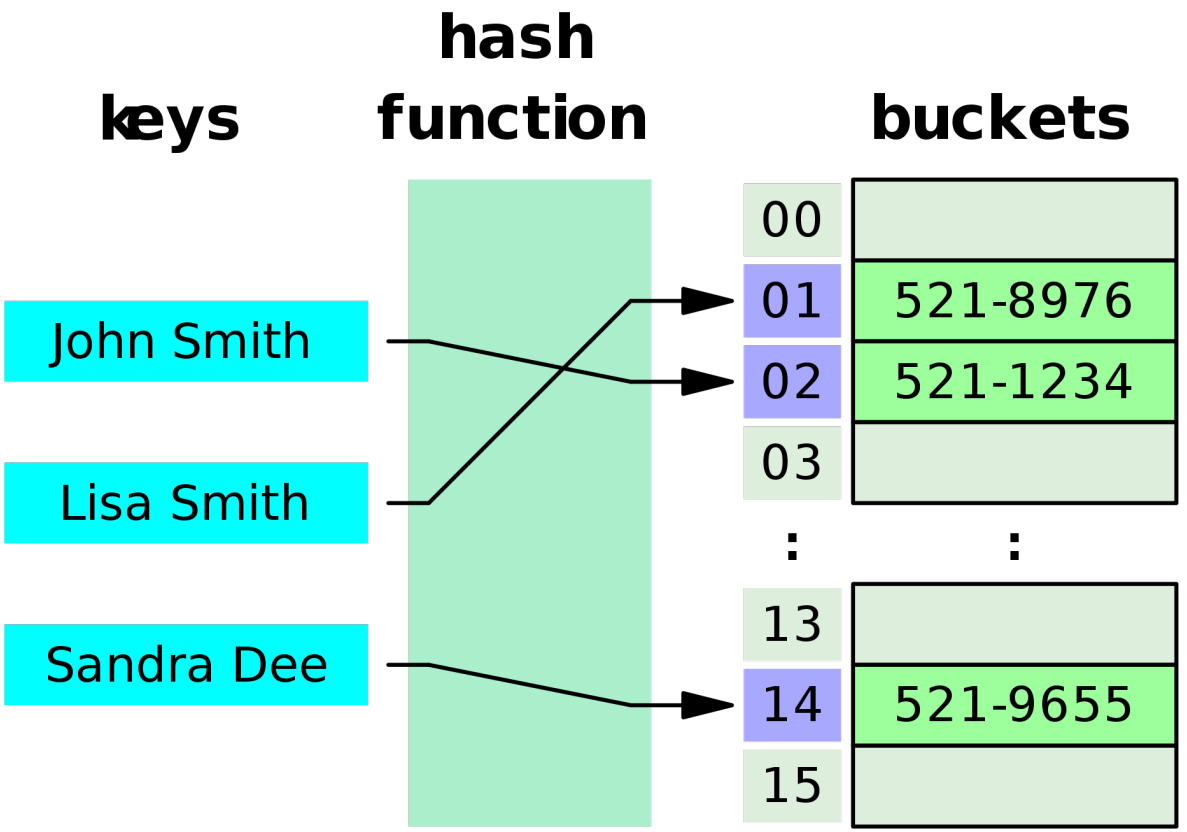
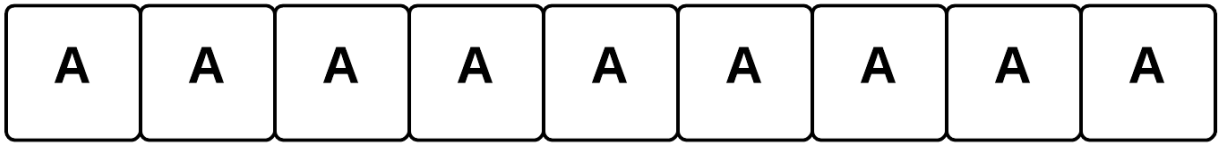
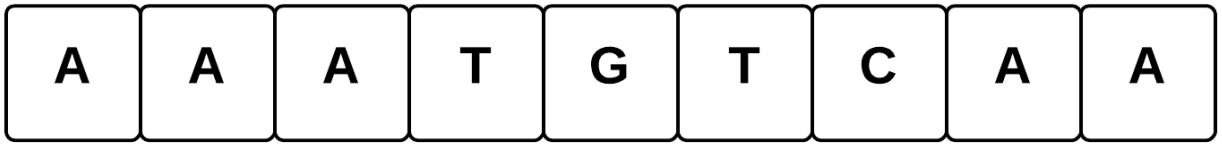


# Hashing sequencing data




**Rule:**

**A-> 00**
**C-> 01**
**G-> 10**
**T-> 11**

# Hashing sequencing data

| A | A | A | A | A | A | A | A | A |
|---|---|---|---|---|---|---|---|---|
| 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

| A | A | A | T | G | T | C | A | A |
|---|---|---|---|---|---|---|---|---|

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# Hashing sequencing data



| A | A | A | A | A | A | A | A | A |
|---|---|---|---|---|---|---|---|---|
| 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

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|---|---|---|---|---|---|---|---|---|
| 00 | 00 | 00 | 11 | 10 | 11 | 01 | 00 | 00 |

**Rule:**

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# Hashing sequencing data



| A | A | A | A | A | A | A | A | A |
|---|---|---|---|---|---|---|---|---|
| 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

= 0

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| A | A | A | T | G | T | C | A | A |
|---|---|---|---|---|---|---|---|---|
| 00 | 00 | 00 | 11 | 10 | 11 | 01 | 00 | 00 |

= 3,792

# Construct hash-table (1)



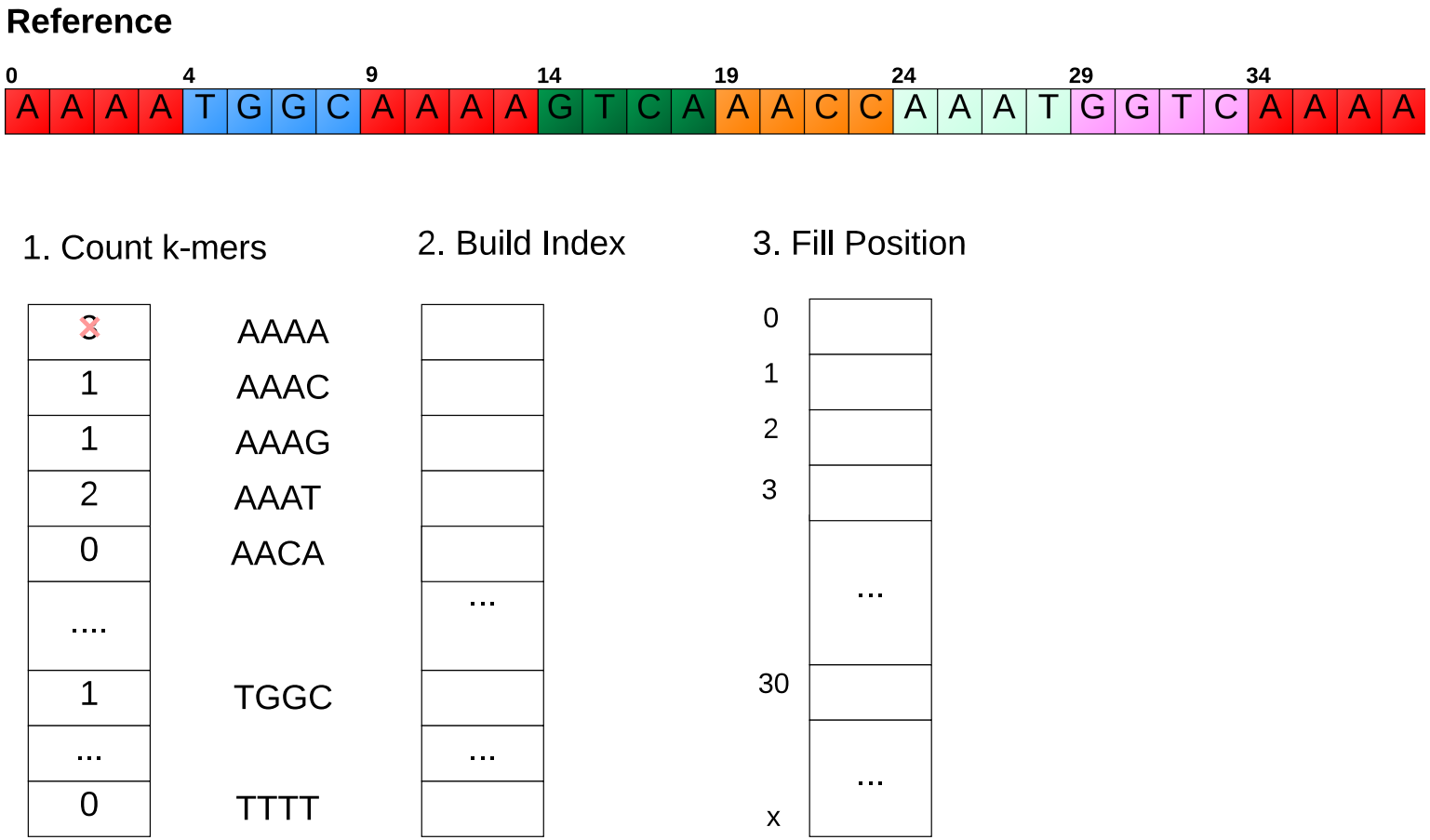
**Reference**


1. Count k-mers

| | |
|---|---|
| 3 | AAAA |
| 1 | AAAC |
| 1 | AAAG |
| 2 | AAAT |
| 0 | AACA |
| .... | |
| 1 | TGGC |
| ... | |
| 0 | TTTT |

# Construct hash-table (2)





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| | |
|---|---|
| ~~3~~ | AAAA |
| 1 | AAAC |
| 1 | AAAG |
| 2 | AAAT |
| 0 | AACA |
| .... | |
| 1 | TGGC |
| ... | |
| 0 | TTTT |

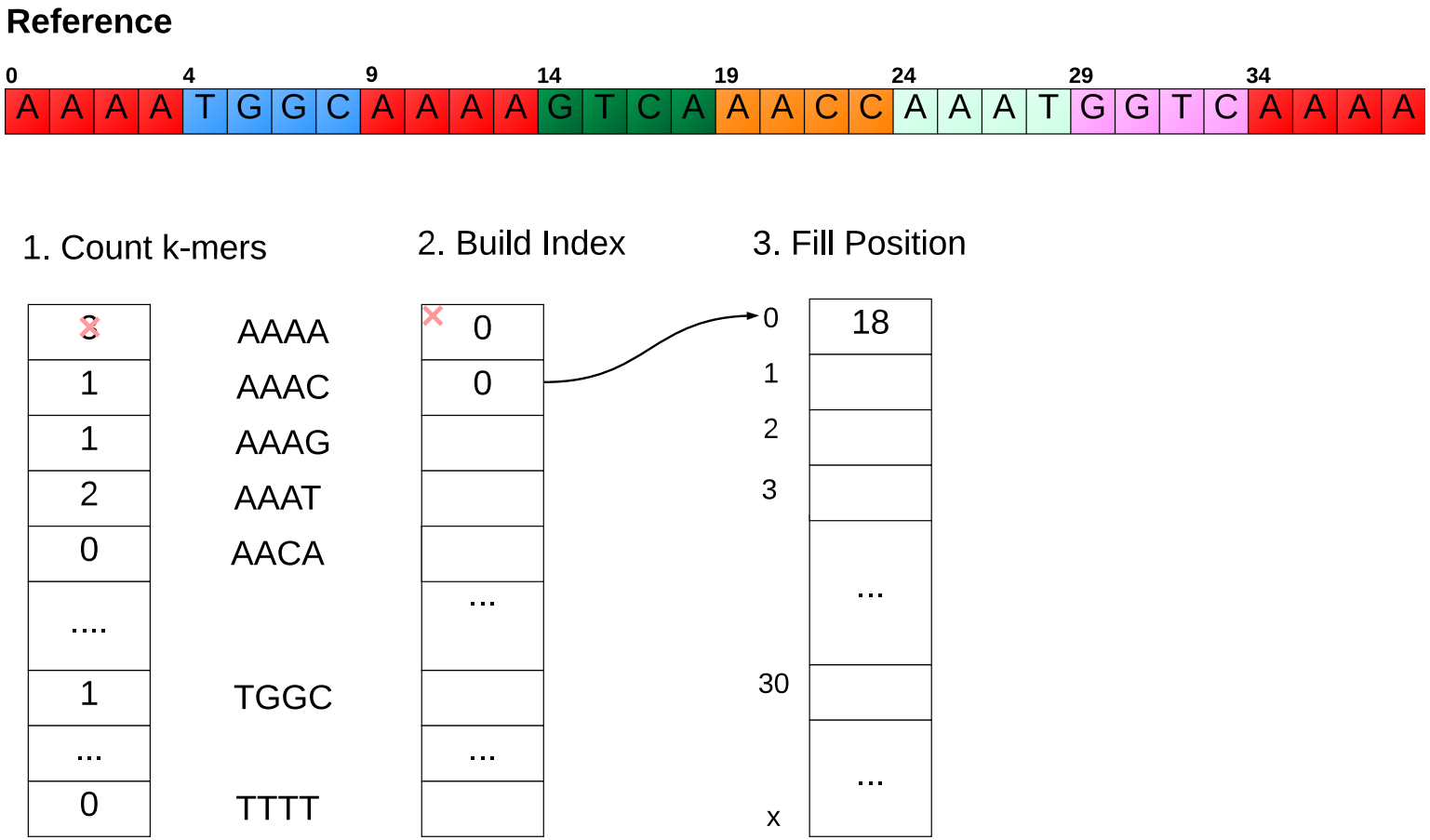
2. Build Index

| |
|---|
| ~~0~~ |
| |
| |
| |
| |
| ... |
| |
| ... |
| |

3. Fill Position

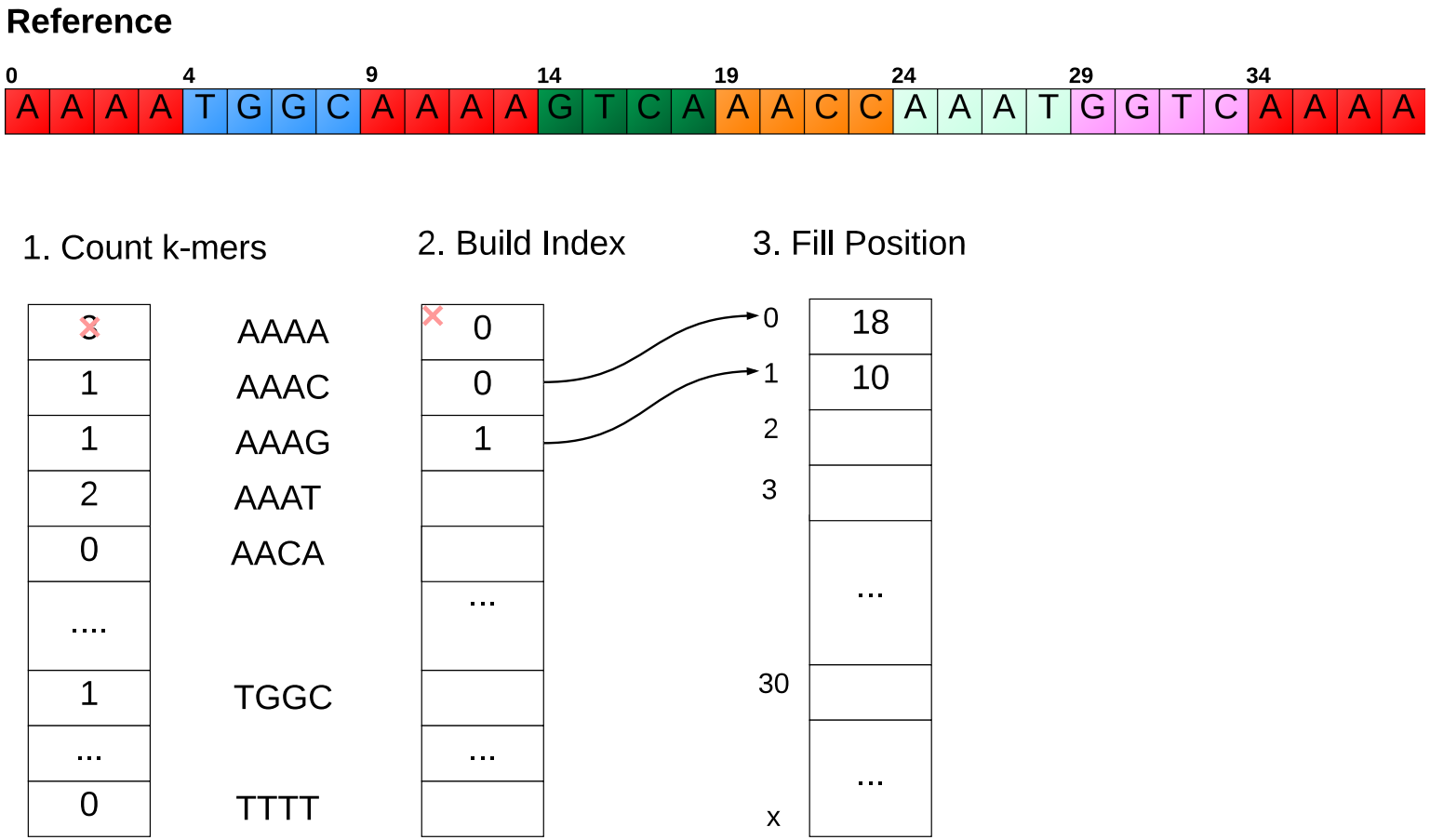
| | |
|---|---|
| 0 | |
| 1 | |
| 2 | |
| 3 | |
| | ... |
| 30 | |
| | ... |
| x | |

# Construct hash-table (2)



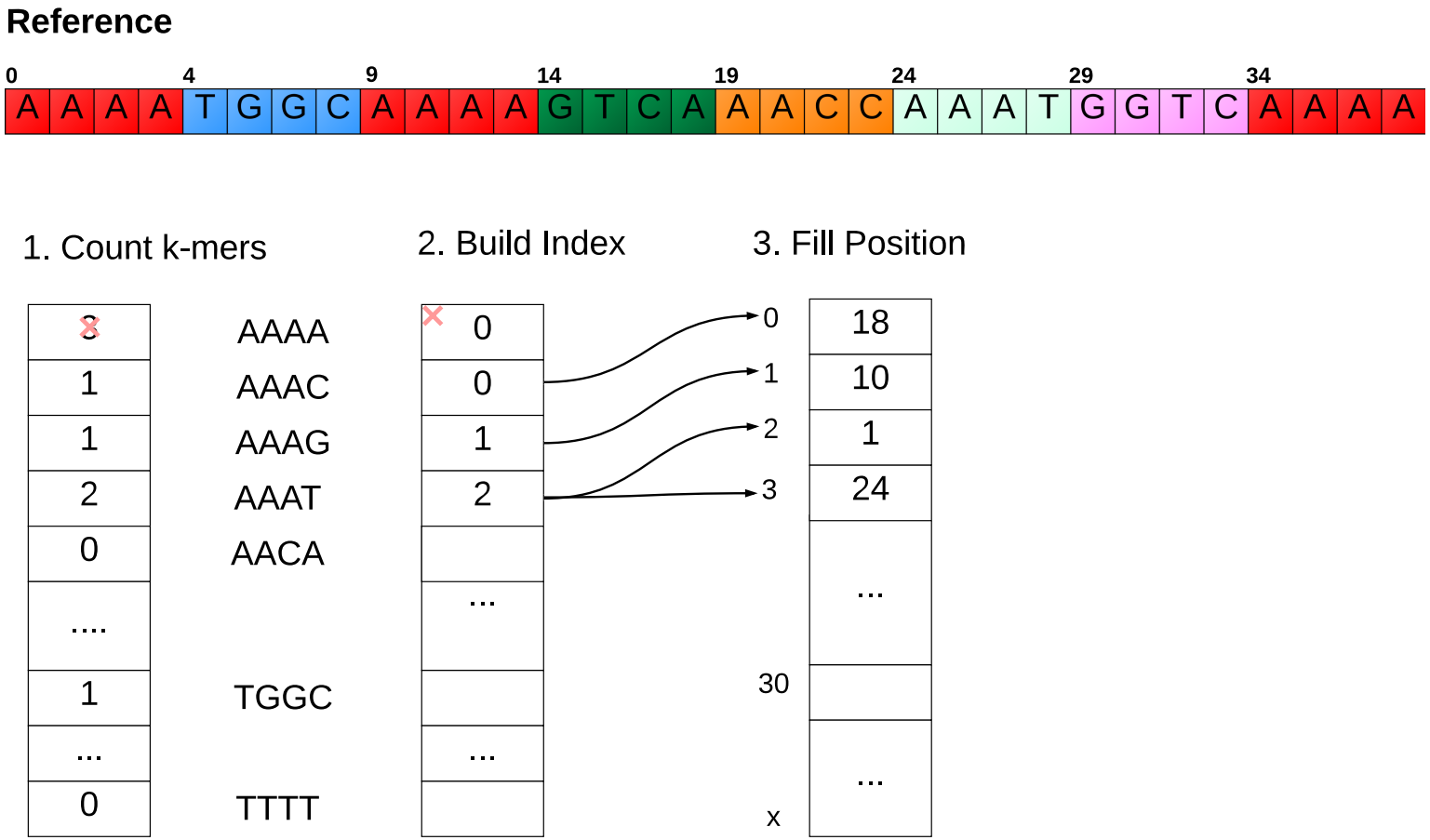
# Construct hash-table (2)



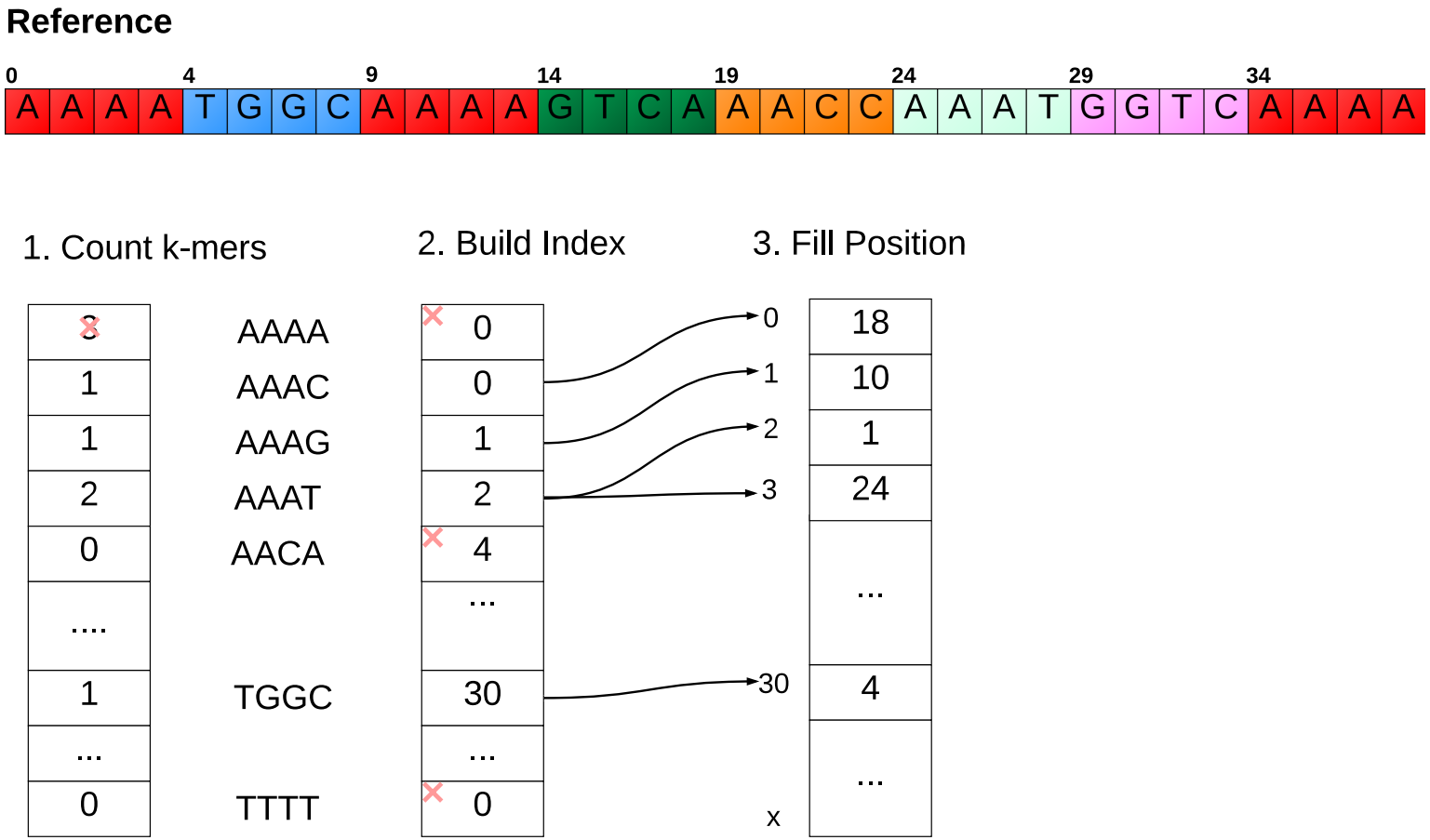


# Construct hash-table (2)





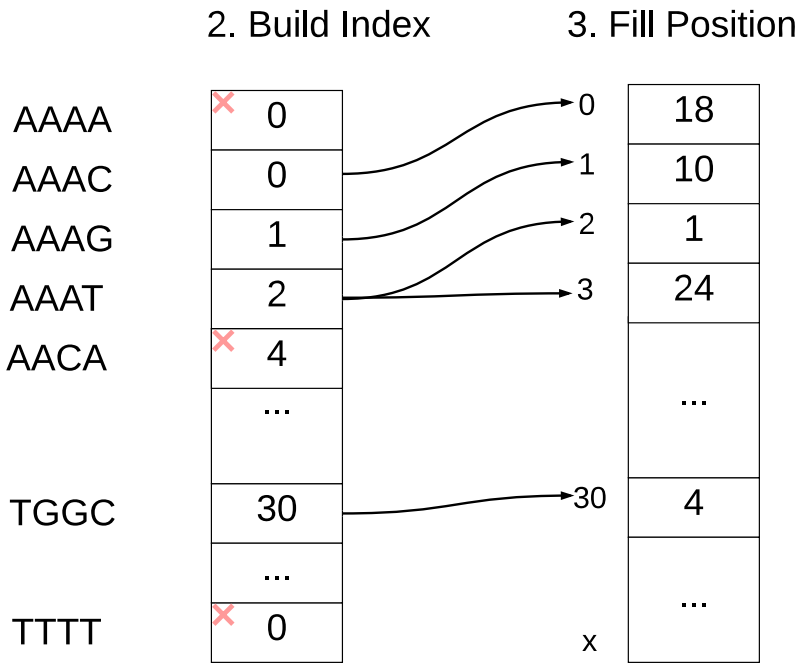
# Construct hash-table (2)



# Construct hash-table (3)

**Advantages:**

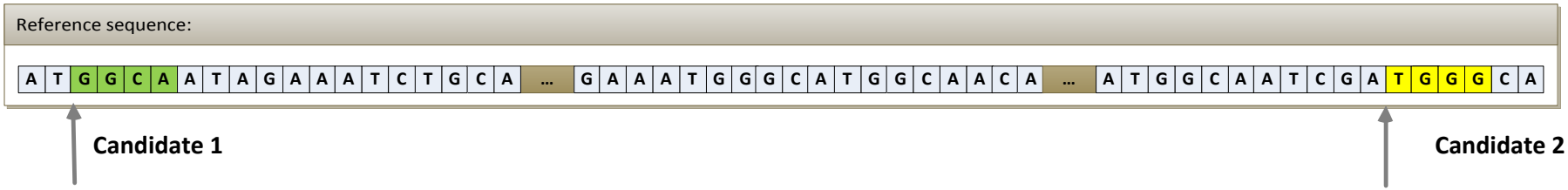
1. Fast access (continuous memory access)
2. Memory efficient ($\sim$ 3 GB for human)

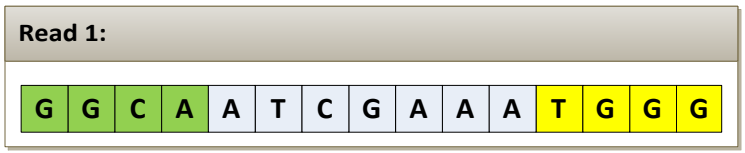


# Candidate region search



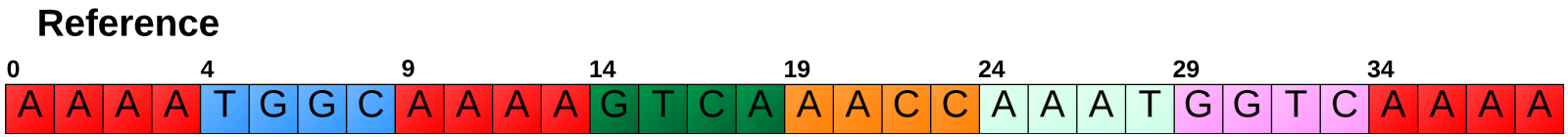
1. Identification of perfectly matching sub regions using an index data structure: **Candidate mapping region (CMR) search**
2. Computation of Smith-Waterman alignment scores for candidates
3. Computation of full alignment only for the highest scoring candidate



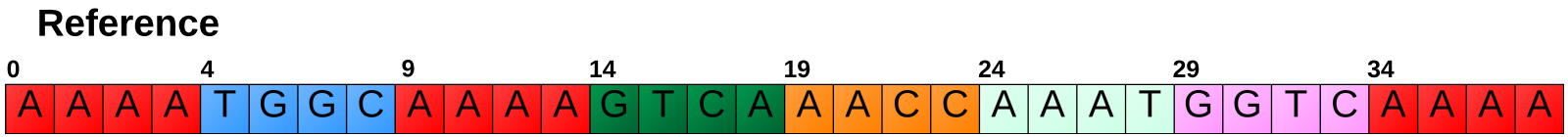



# Collect candidate regions





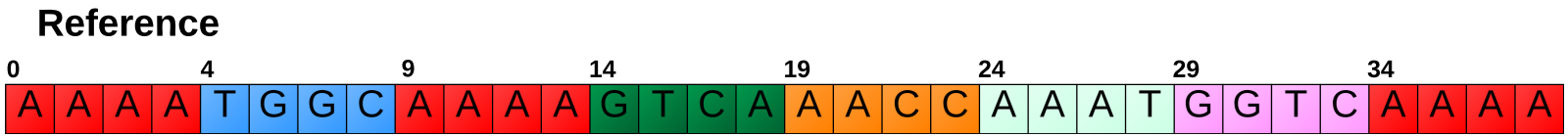
# Collect candidate regions




| K-mers | genomic positions |
|---|---|
| A A A A | |
| A A A T | 1, 24 |
| A A T G | 2, 25 |
| A T G G | 3, 26 |
| T G G C | 4 |

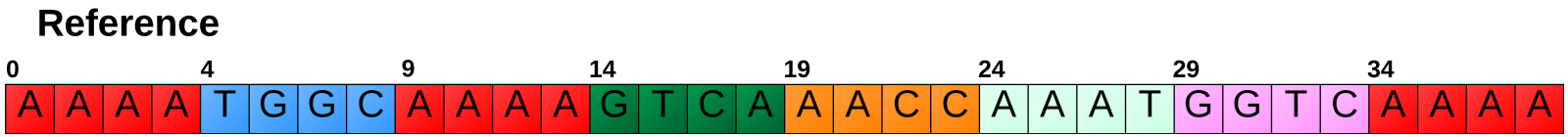
| K-mers | genomic positions |
|---|---|
| A A A T | 1, 24 |
| A A T G | 2, 25 |
| A T G T | |
| T G T C | |
| G T C A | 14, 30 |

# Correct candidate regions




| K-mers | genomic positions | | K-mers | genomic positions | |
|---|---|---|---|---|---|
| A A A A | | -0 | A A A T | 1, 24 | -0 |
| A A A T | 1, 24 | -1 | A A T G | 2, 25 | -1 |
| A A T G | 2, 25 | -2 | A T G T | | -2 |
| A T G G | 3, 26 | -3 | T G T C | | -3 |
| T G G C | 4 | -4 | G T C A | 14, 30 | -4 |

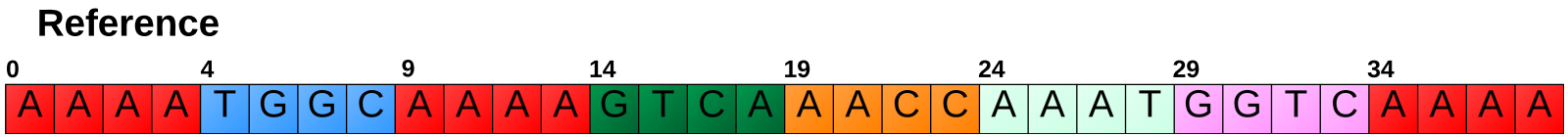
# Correct candidate regions




| K-mers | genomic positions |
|---|---|
| A A A A | |
| A A A T | 0, 23 |
| A A T G | 0, 23 |
| A T G G | 0, 23 |
| T G G C | 0 |

| K-mers | genomic positions |
|---|---|
| A A A T | 1, 24 |
| A A T G | 1, 24 |
| A T G T | |
| T G T C | |
| G T C A | 9, 25 |

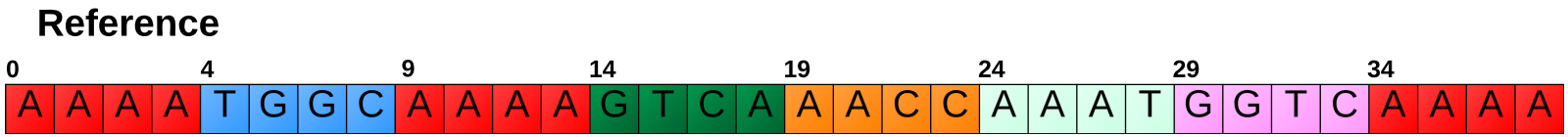
# Account for indels

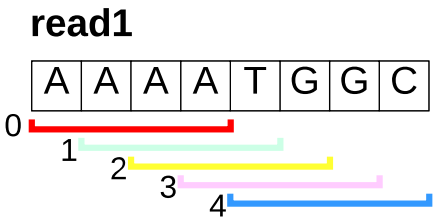


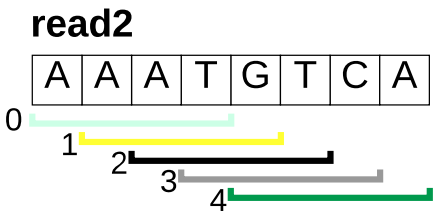

| K-mers | genomic positions | | K-mers | genomic positions | |
|---|---|---|---|---|---|
| A A A A | | /8 | A A A T | 1, 24 | /8 |
| A A A T | 0, 23 | /8 | A A T G | 1, 24 | /8 |
| A A T G | 0, 23 | /8 | A T G T | | /8 |
| A T G G | 0, 23 | /8 | T G T C | | /8 |
| T G G C | 0 | /8 | G T C A | 9, 25 | /8 |

# Account for indels





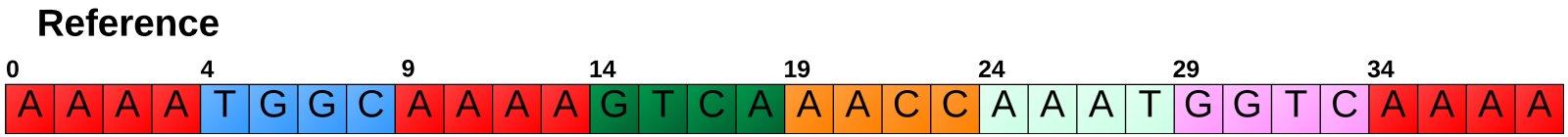



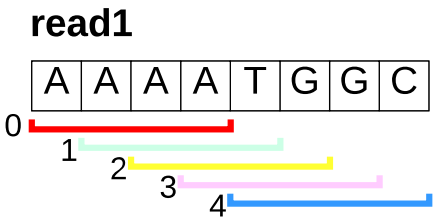

| K-mers | genomic positions |
|---|---|
| A A A A | |
| A A A T | 0, 2 |
| A A T G | 0, 2 |
| A T G G | 0, 2 |
| T G G C | 0 |

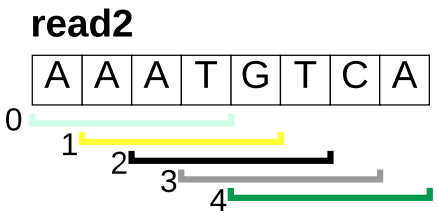
| K-mers | genomic positions |
|---|---|
| A A A T | 0, 3 |
| A A T G | 0, 3 |
| A T G T | |
| T G T C | |
| G T C A | 1, 3 |

# Account for indels





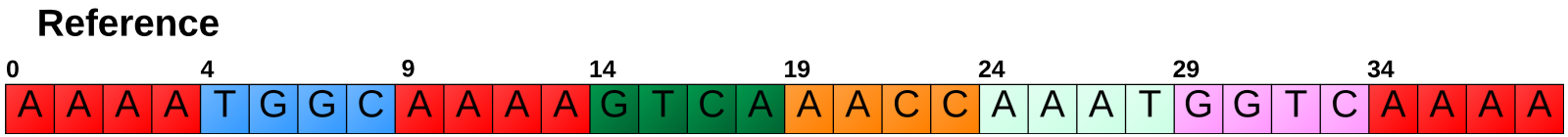



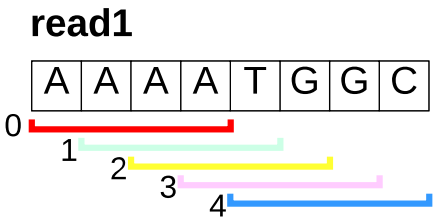



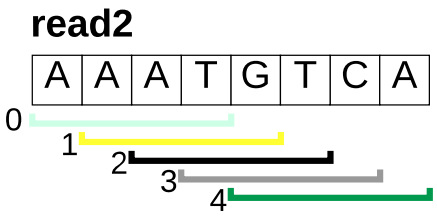

| K-mers | genomic positions | |
|---|---|---|
| A A A A | | *8 |
| A A A T | 0, 2 | *8 |
| A A T G | 0, 2 | *8 |
| A T G G | 0, 2 | *8 |
| T G G C | 0 | *8 |

| K-mers | genomic positions | |
|---|---|---|
| A A A T | 0, 3 | *8 |
| A A T G | 0, 3 | *8 |
| A T G T | | *8 |
| T G T C | | *8 |
| G T C A | 1, 3 | *8 |

# Account for indels




| K-mers | genomic positions |
|---|---|
| A A A A | |
| A A A T | 0, 16 |
| A A T G | 0, 16 |
| A T G G | 0, 16 |
| T G G C | 0 |

| K-mers | genomic positions |
|---|---|
| A A A T | 0, 24 |
| A A T G | 0, 24 |
| A T G T | |
| T G T C | |
| G T C A | 8, 24 |

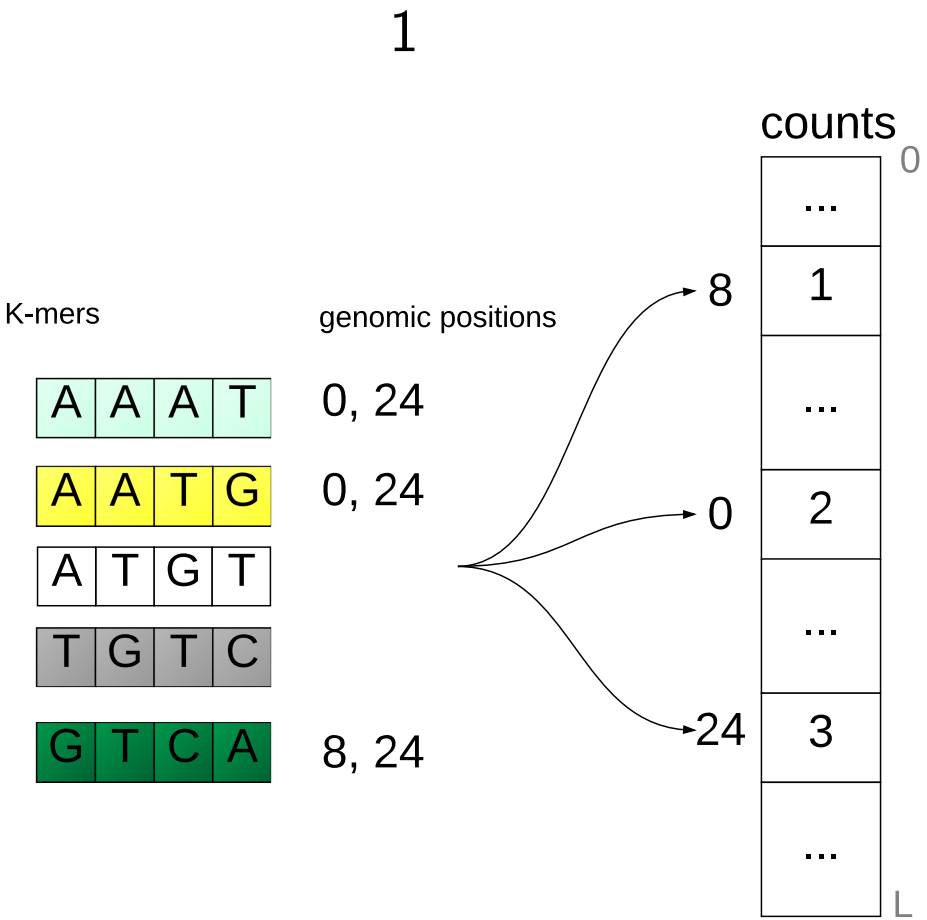
# Summarize candidate regions

**Multiplication hashing**

$$key_j = (p'_j * prim) \bmod L$$

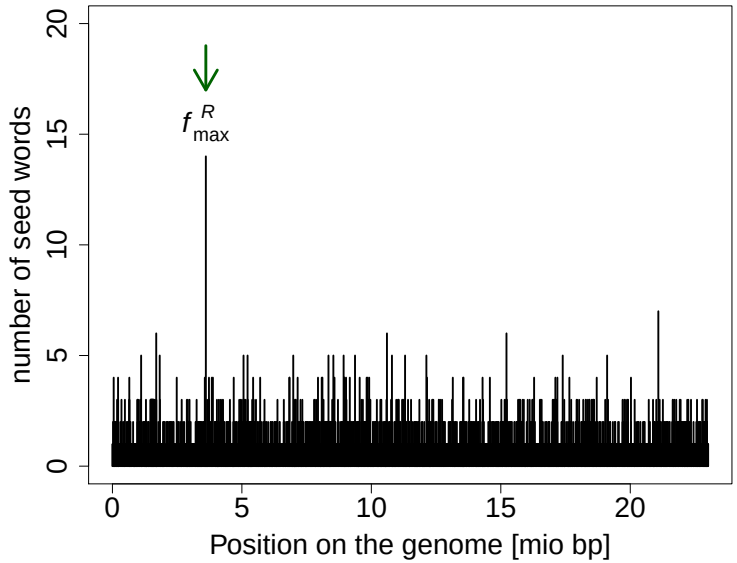
$$prim = 2654435761$$

Donald E. Knuth,1997



# Choosing candidate regions

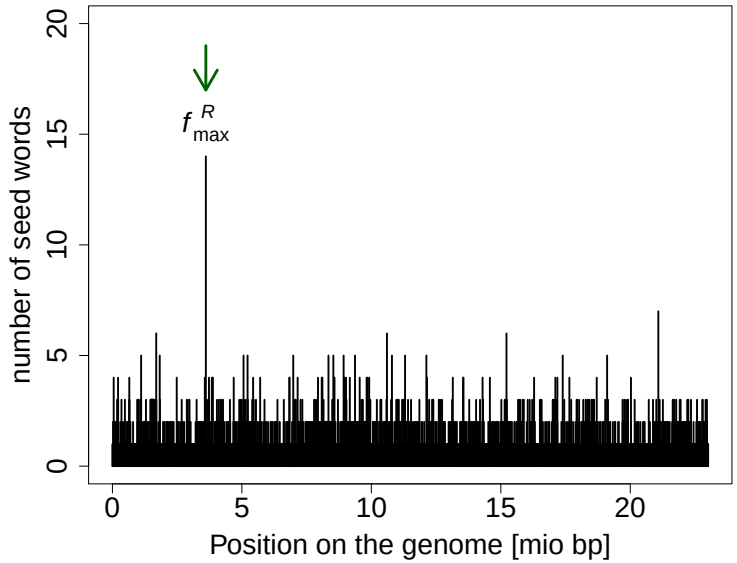


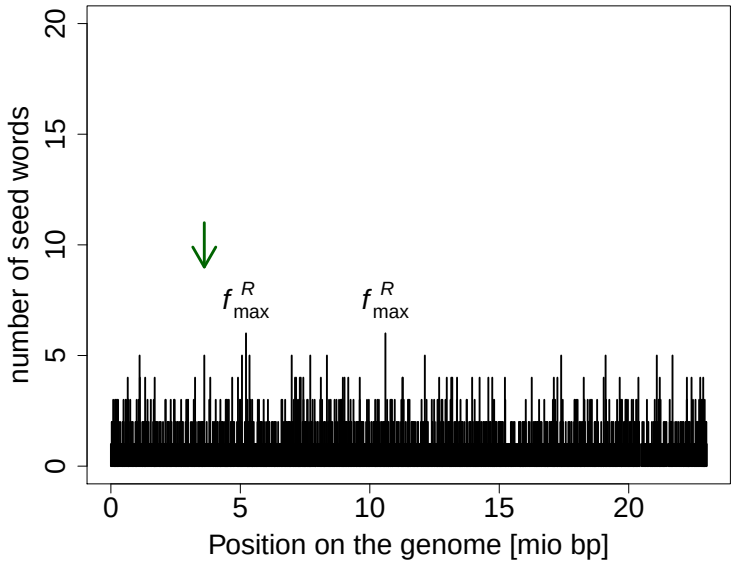



- Fixed threshold ($t = 2$) for all reads: 1614 alignment computations
- Choose $t$ relative to best matching position: 214 alignment computations

# Choosing candidate regions



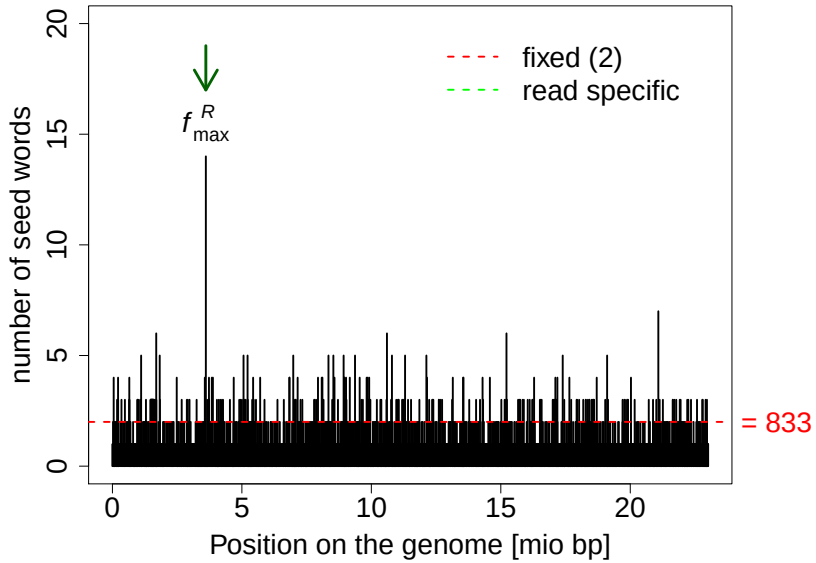


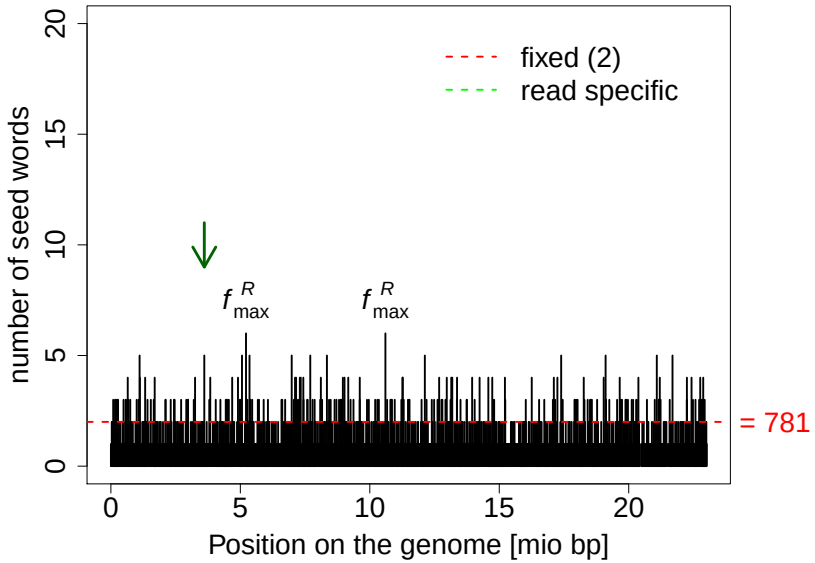




- Fixed threshold ($t = 2$) for all reads: 1614 alignment computations
- Choose $t$ relative to best matching position: 214 alignment computations

# Choosing candidate regions



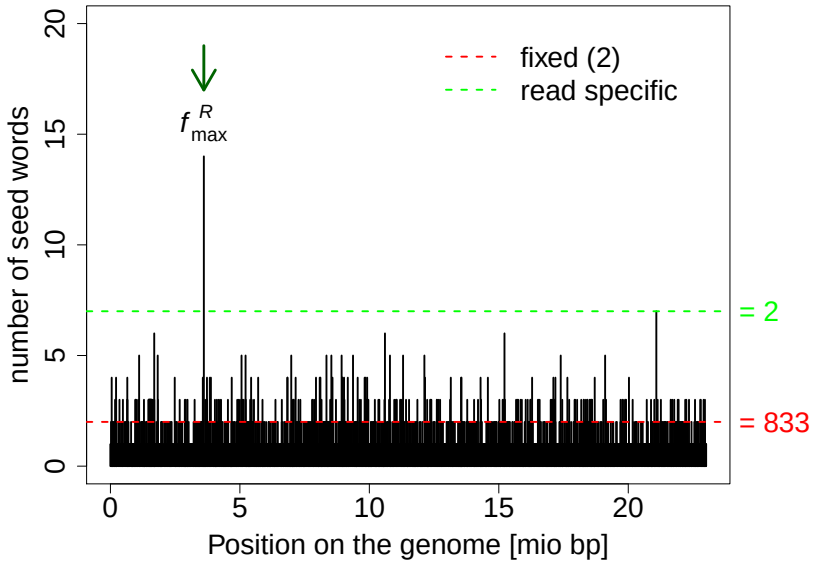


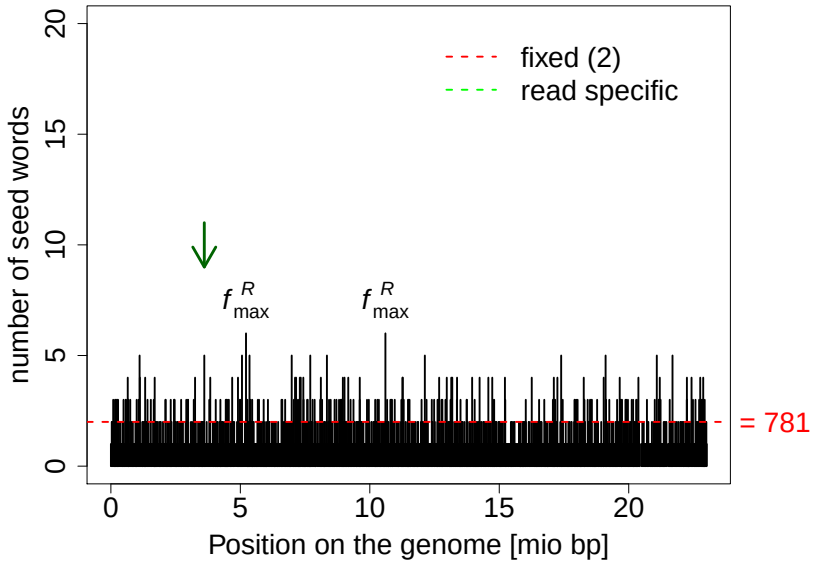




- Fixed threshold ($t = 2$) for all reads: 1614 alignment computations
- Choose $t$ relative to best matching position: 214 alignment computations

# Choosing candidate regions



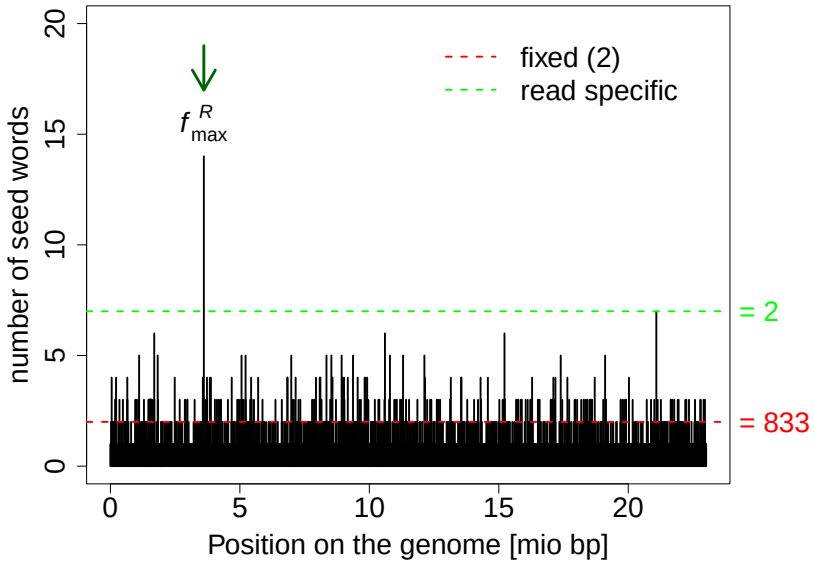


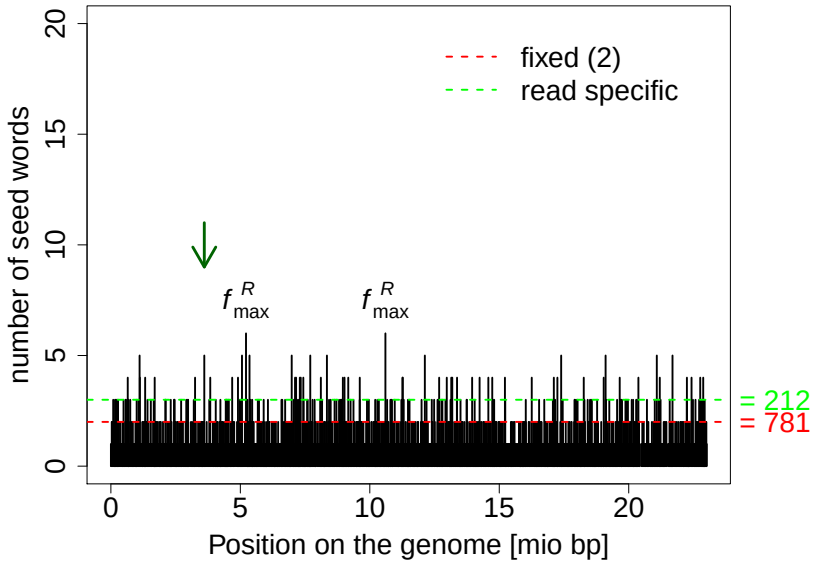




- Fixed threshold ($t = 2$) for all reads: 1614 alignment computations
- Choose $t$ relative to best matching position: 214 alignment computations

# Choosing candidate regions





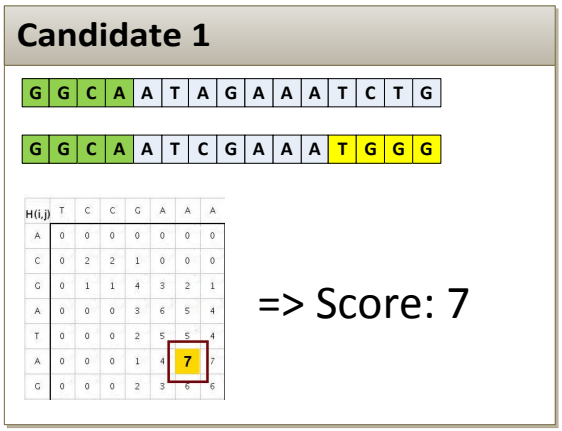



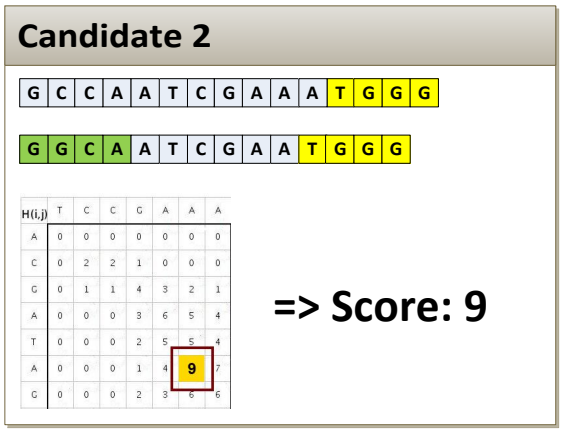

- Fixed threshold ($t = 2$) for all reads: 1614 alignment computations
- Choose $t$ relative to best matching position: 214 alignment computations

# Step 2 & 3: alignment computation



1. Candidate mapping region search: identification of perfectly matching sub regions using an index data structure
2. Computation of Smith-Waterman alignment scores for candidates
3. Computation of full alignment only for the highest scoring candidate



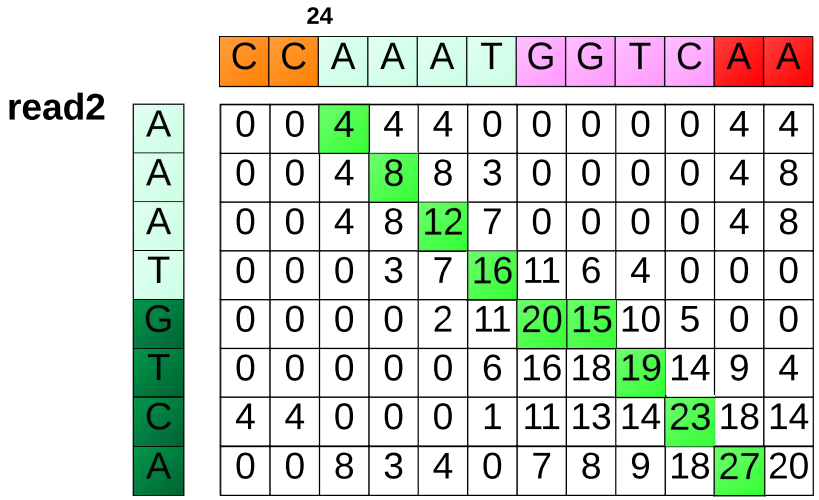

...

# Pairwise alignment

Complexity: $n \times m$

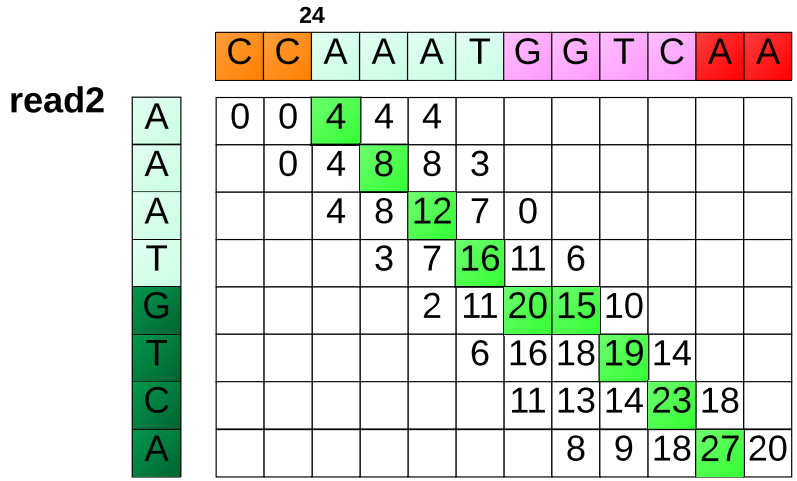
$n = 120bp$

$m = 100bp$



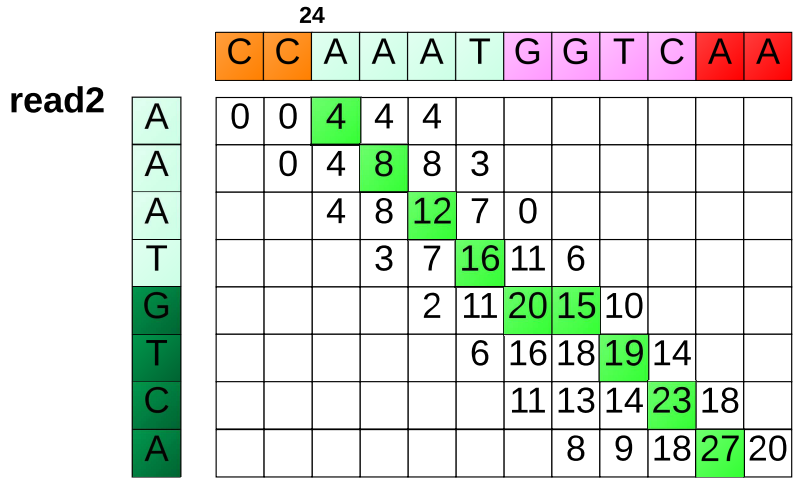
# Algorithmic optimizations

1. Banded alignment
   Complexity: $m \times c$
   $m = 100bp$
   $c \in \{1, \ldots, 40\}$
2. Split score and alignment computation
3. For score computation
   Computations, $m \times c$
   Memory, $c$



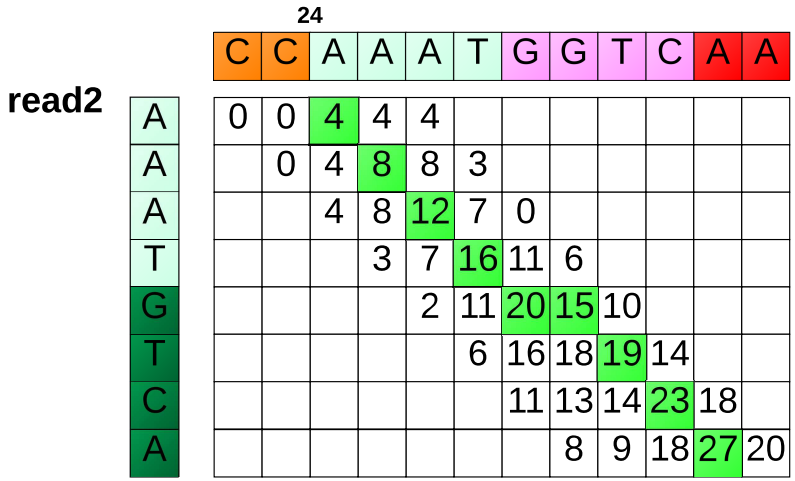
# Algorithmic optimizations

1. Banded alignment
   Complexity: $m \times c$
   $m = 100bp$
   $c \in \{1, \ldots, 40\}$
2. Split score and alignment computation
3. For score computation
   Computations, $m \times c$
   Memory, $c$

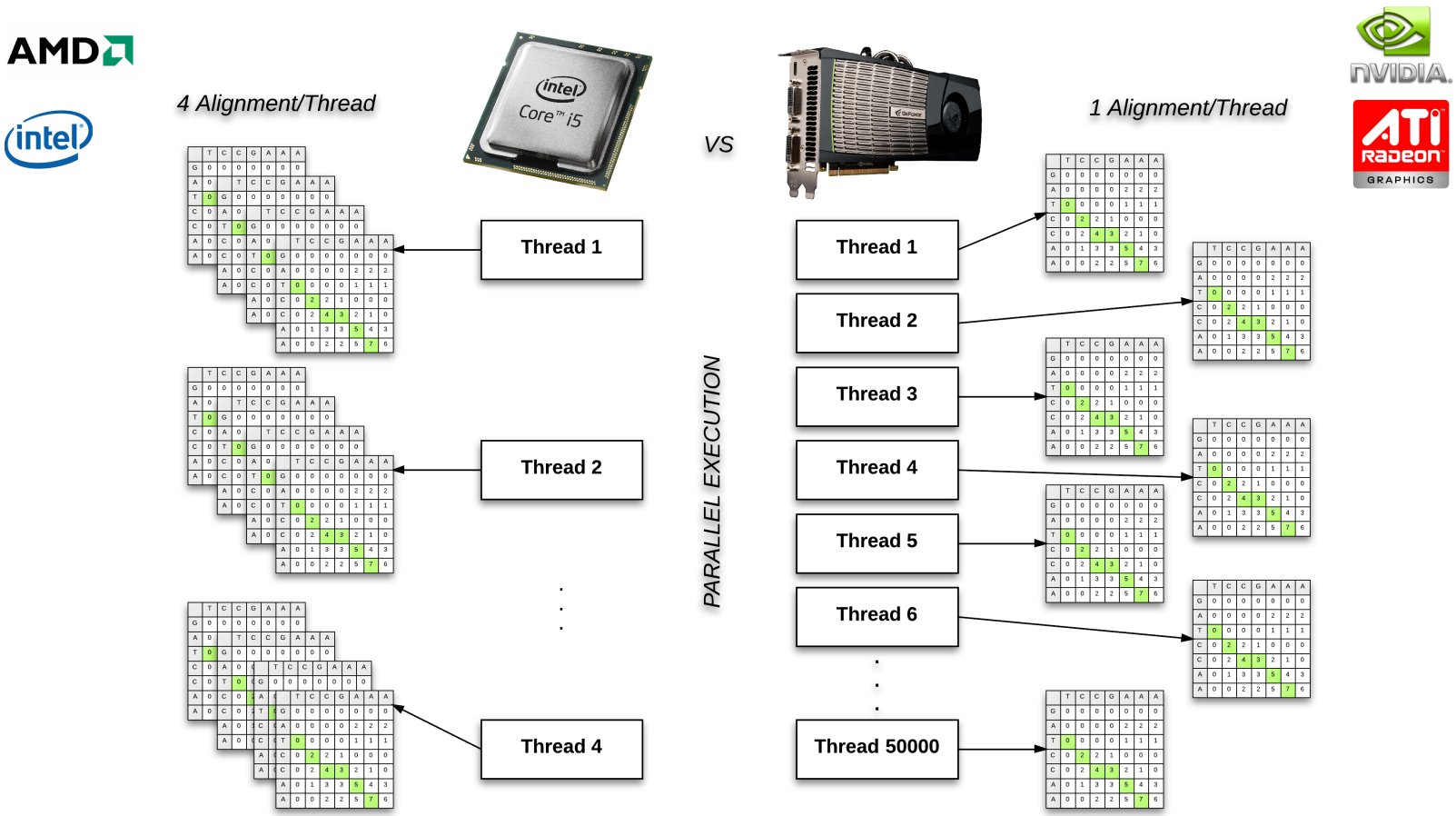


# Algorithmic optimizations

1. Banded alignment
   Complexity: $m \times c$
   $m = 100bp$
   $c \in \{1, \ldots, 40\}$
2. Split score and alignment computation
3. For score computation
   Computations, $m \times c$
   Memory, $c$



# Hardware optimizations

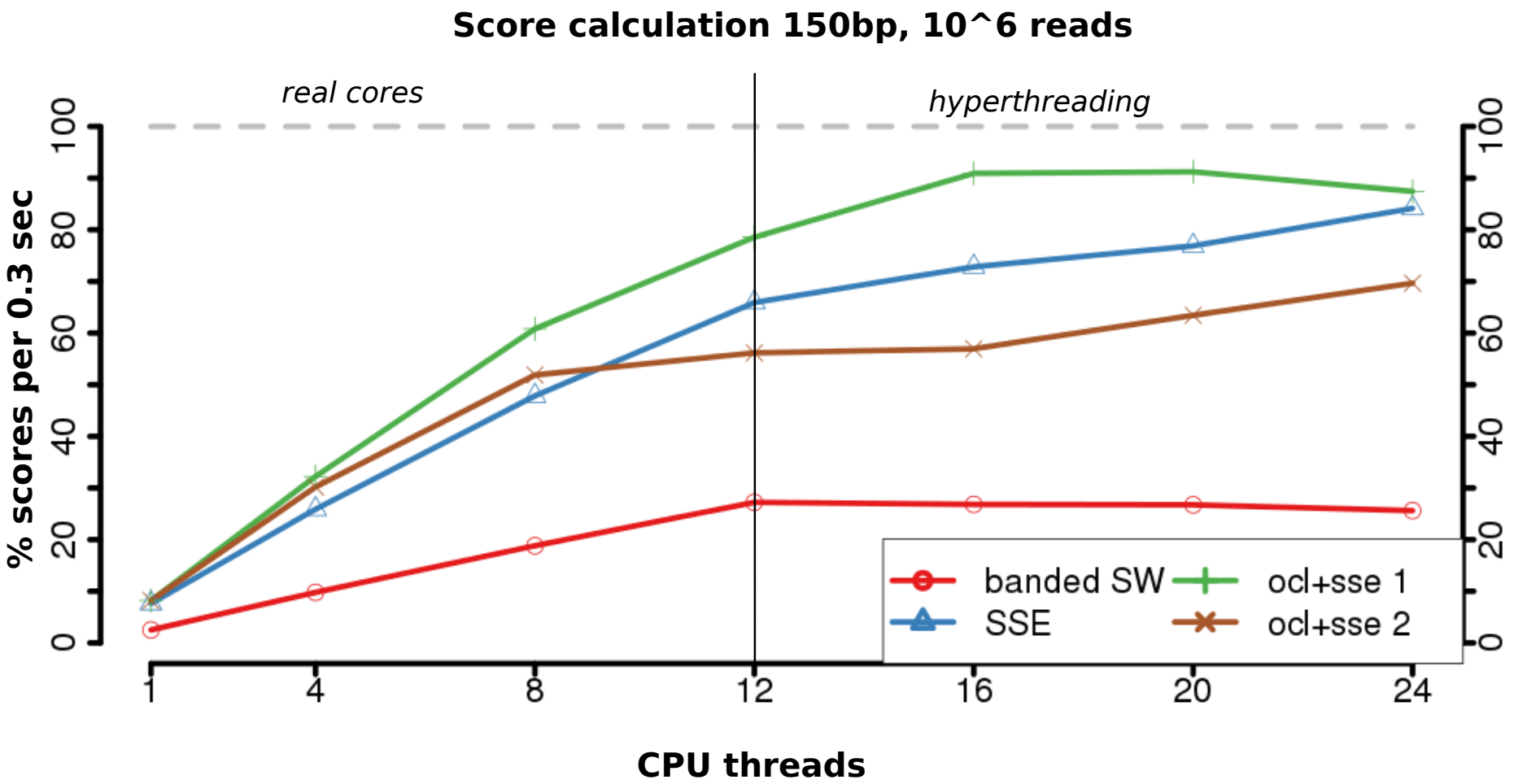



| OpenCL | |
|---|---|
| Hardware: | Intel i5-2500k |
| Sequences: | 1 million |
| Time (Scores): | 0.48 s |
| Time (Alignmets): | 2.18 s |

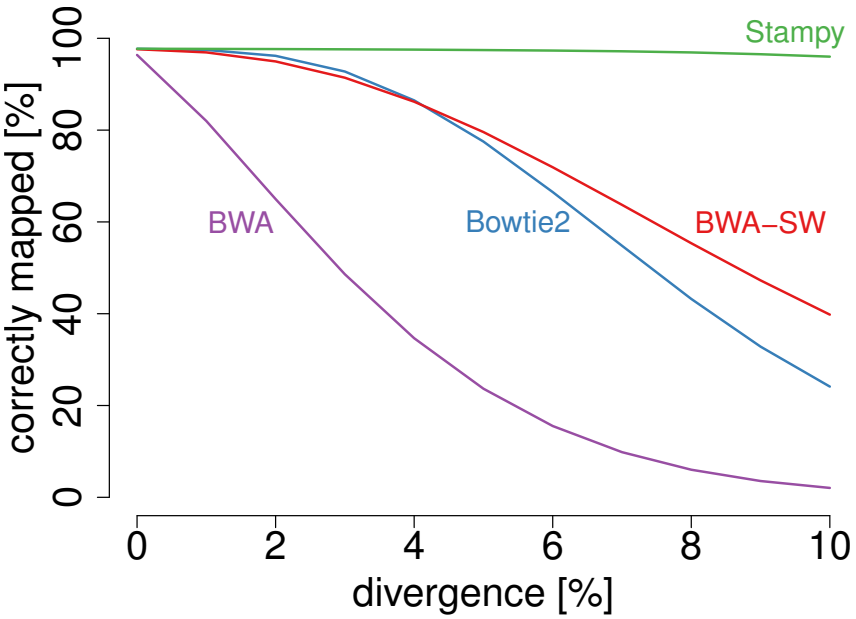
OpenCL

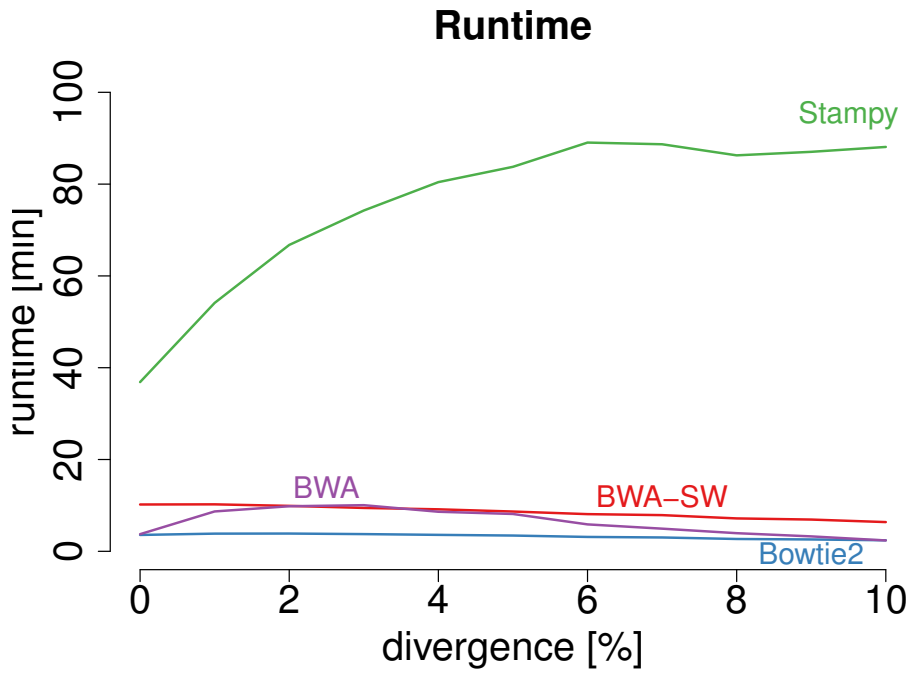
| OpenCL | |
|---|---|
| Hardware: | Nvidia GTX480 |
| Sequences: | 1 million |
| Time (Scores): | 0.25 s |
| Time (Alignmets): | 1.14 s |

# Hardware optimizations

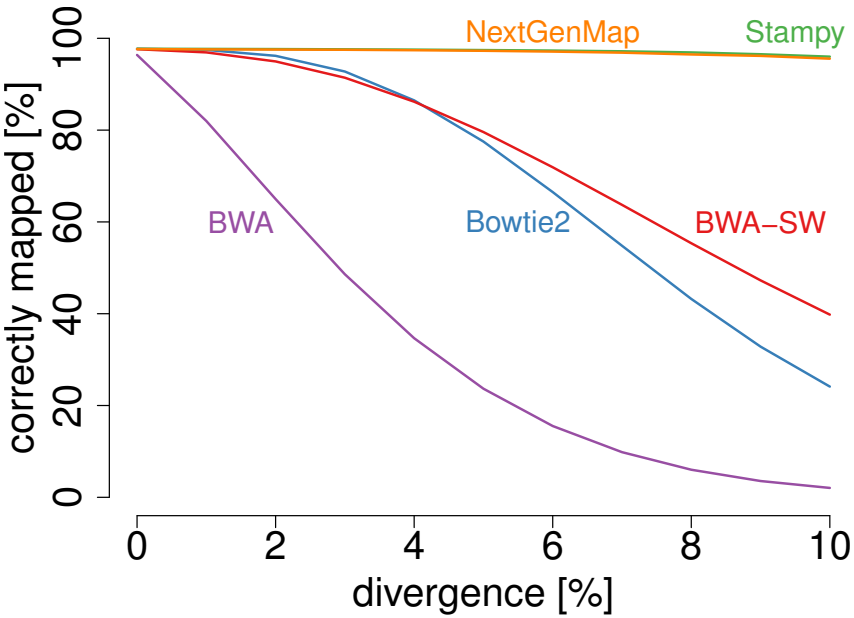


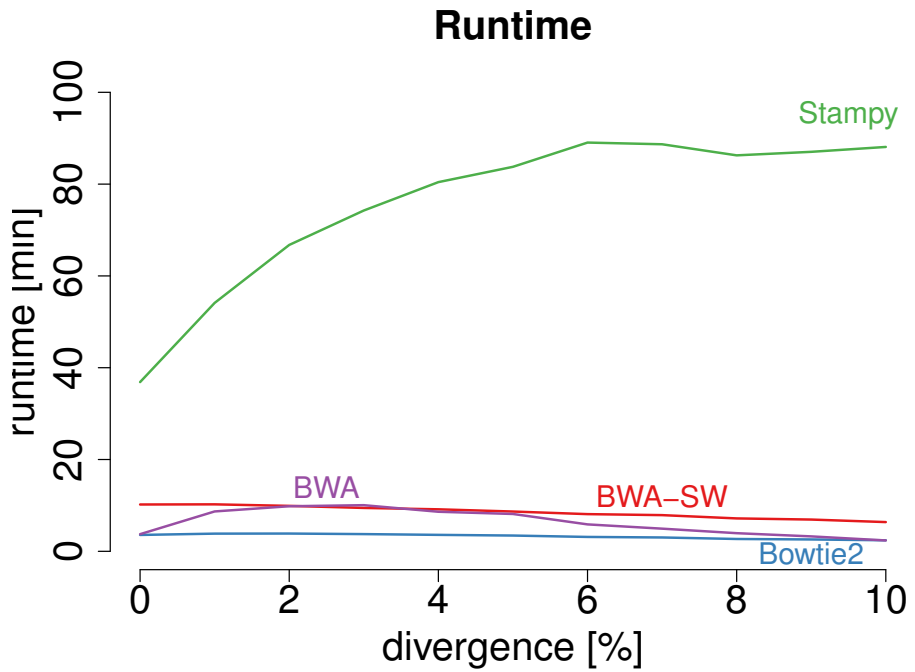
# Results


correctly mapped [%]
0
20
40
60
80
100
0
2
4
6
8
10
divergence [%]
Stampy
BWA
Bowtie2
BWA–SW

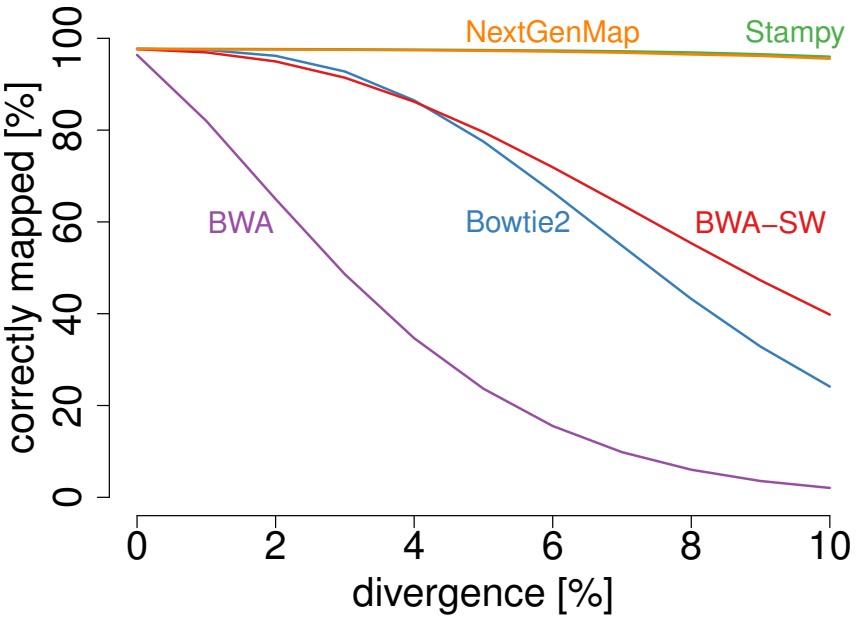

Runtime
runtime [min]
0
20
40
60
80
100
0
2
4
6
8
10
divergence [%]
Stampy
BWA
BWA–SW
Bowtie2

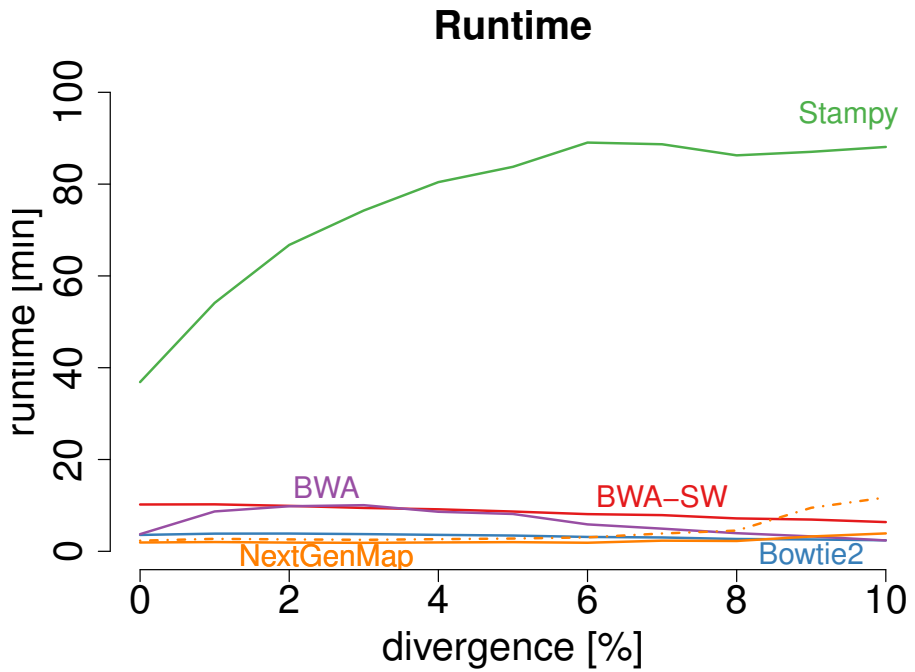
# Results


NextGenMap
Stampy
BWA
Bowtie2
BWA–SW
correctly mapped [%]
0
20
40
60
80
100
0
2
4
6
8
10
divergence [%]


Runtime
Stampy
BWA
BWA–SW
Bowtie2
runtime [min]
0
20
40
60
80
100
0
2
4
6
8
10
divergence [%]

# Results


NextGenMap
Stampy
BWA
Bowtie2
BWA–SW
correctly mapped [%]
0
20
40
60
80
100
0
2
4
6
8
10
divergence [%]


Runtime
Stampy
BWA
BWA–SW
NextGenMap
Bowtie2
runtime [min]
0
20
40
60
80
100
0
2
4
6
8
10
divergence [%]

# Applications



- Finding sRNAs in borrelia
- Identifying mutations in tumor tissues
- Sequencing based test for sepsis in patients

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# Conclusion



**Read mapping:**

1. Finding the region of the reference genome that shows the highest similarity to a given read
2. It is one of the central steps in NGS data analysis
3. Size of genome, number of reads and repeating regions make it difficult to find the correct mapping location in reasonable time
4. Several approaches: sequence alignments (optimal), seed and extend (heuristic), tree based methods (heuristic)

**Seed and extend methods:**

1. Hash-table is used to quickly identify regions on the genome that are similar to a given read
2. Sequences alignments are used to find the region that shows the highest similarity to a read
3. Exploiting modern hardware reduces runtime required for alignment computations significantly