

INTRODUCTION TO (GENOMIC) ALIGNMENTS

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1. Background
2. Exact String Matching Algorithms
 - 2.1. Brute Force String Matching
 - 2.2. Boyer-Moore String Matching
 - 2.3. K-mer Indexes
3. A Bioinformatic Example
4. Approximate String Matching Algorithms
 - 4.1. Hamming Distance & Edit Distance
 - 4.2. Global Alignment
 - 4.3. Local Alignment
5. A True To Life Example
6. Teaching Material

1. BACKGROUND



Illumina Nextera DNA
Flex Library Prep Kit



Invitrogen QuBit



Illumina HiSeq 4000

*SAMPLE
PREPARATION*

*SAMPLE
QUANTIFICATION*

*SAMPLE
SEQUENCING*

*SEQUENCING
READS*



Oxford Nanopore
Technologies DNA
sequencing kit



Invitrogen QuBit



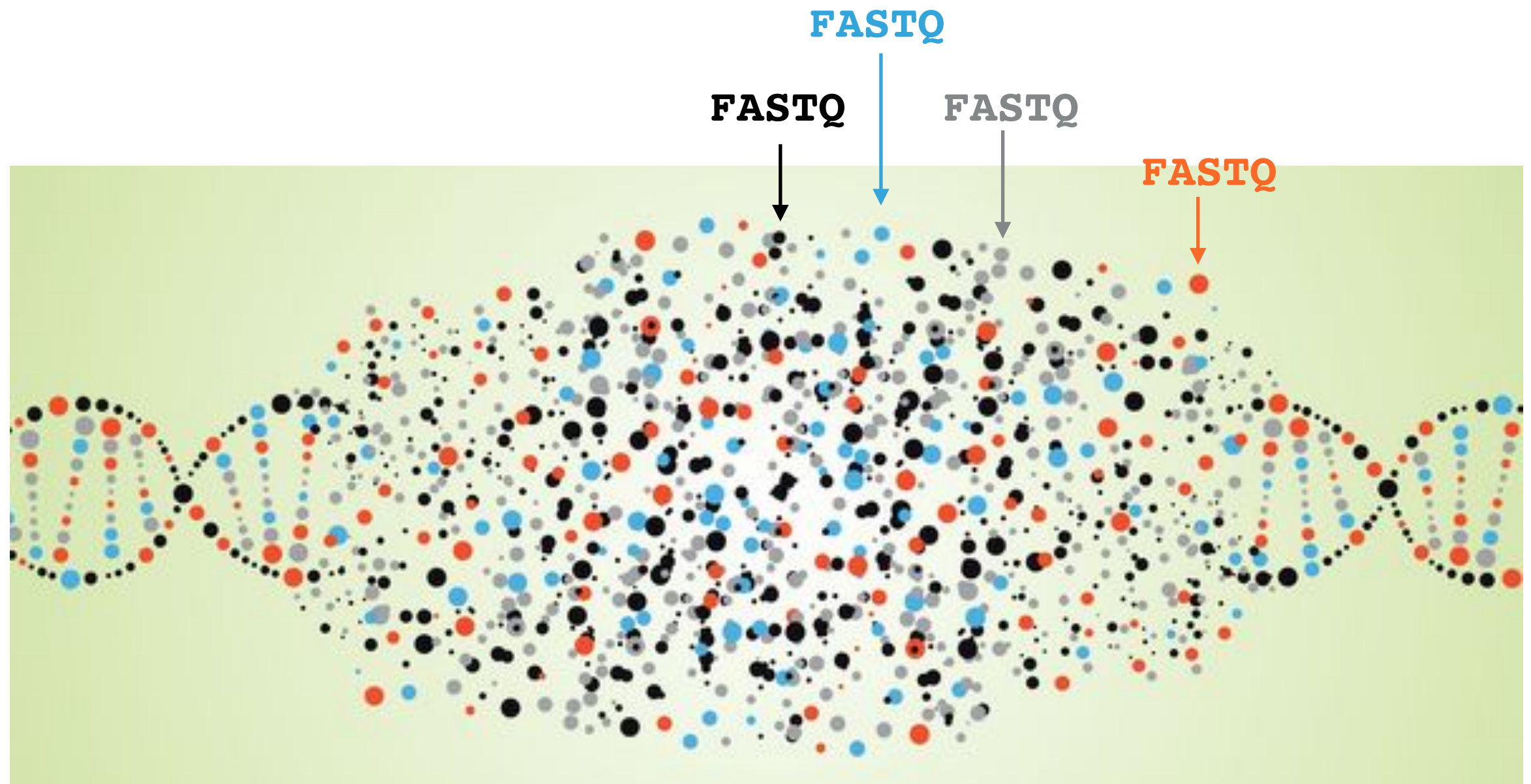
Oxford Nanopore
Technologies GridION X5

READ: FASTQ

```
@SEQ_ID @ Sequence ID
TGCCCAGTGGTGGAGGTCTCAGAAATGGTATAAAAGTCAGACAAATCTTGGTTTTGCATT Sequence
++ (Sequence ID)
!'**((( (**+))%%%++) (%%%) .1***-+*'') **55CCF>>>>>CCCCCCC65 Sequence quality
```

REFERENCE: FASTA

```
>chr20:17000000-17000539 > Sequence ID
AGAGTGAGACTCTGAAATAAATAAATAAATAAATAAACAATAAACCACGAGCAAGA
GACAAACAGGAAAAAAAAAAAAAGAATGAACAATTATTTATTTTGGGAAGAAACATCTGAGAT
GGTAGGTAGTGTCAAAGATAGCAGTGCCCAGTGGTGGAGGTCTCAGAAATGGTATAAAAG
TCAGACAAATCTTGGTTTTTGCATTCTCCAAGTCTGGTTTTTTTAGCCTCTGCTGACTGCTG
GAACTACTTGATAATCTTCCAAAAATTACTTCTTGACTAAGGTCACCAAGGCCAGTTTTT Sequence
GTGGCTTGCACCTGGACTCTTGGCTGGCACTGGGACATGCTGCTTAATGGCGGGAAAACA
ATTAATAATTCCCATTTAAGTCAGGAAAAATACAAGATTGTATATCACCATTACTATTTT
TAGCATTGTCCTGGAAGTAAAATGTAATTATACAAGAAGATTATGTTTGGTTTACATATT
GAAAAGAAGGCAGAAAATTTATCATTATTTGTAGACCATCCTATTATCTACCCAGAAGAT
```



The genome jigsaw

FASTA

Take-home message #1

Genomic alignments map the sequencing reads to the reference genome in order to identify their original genomic *loci*

READ

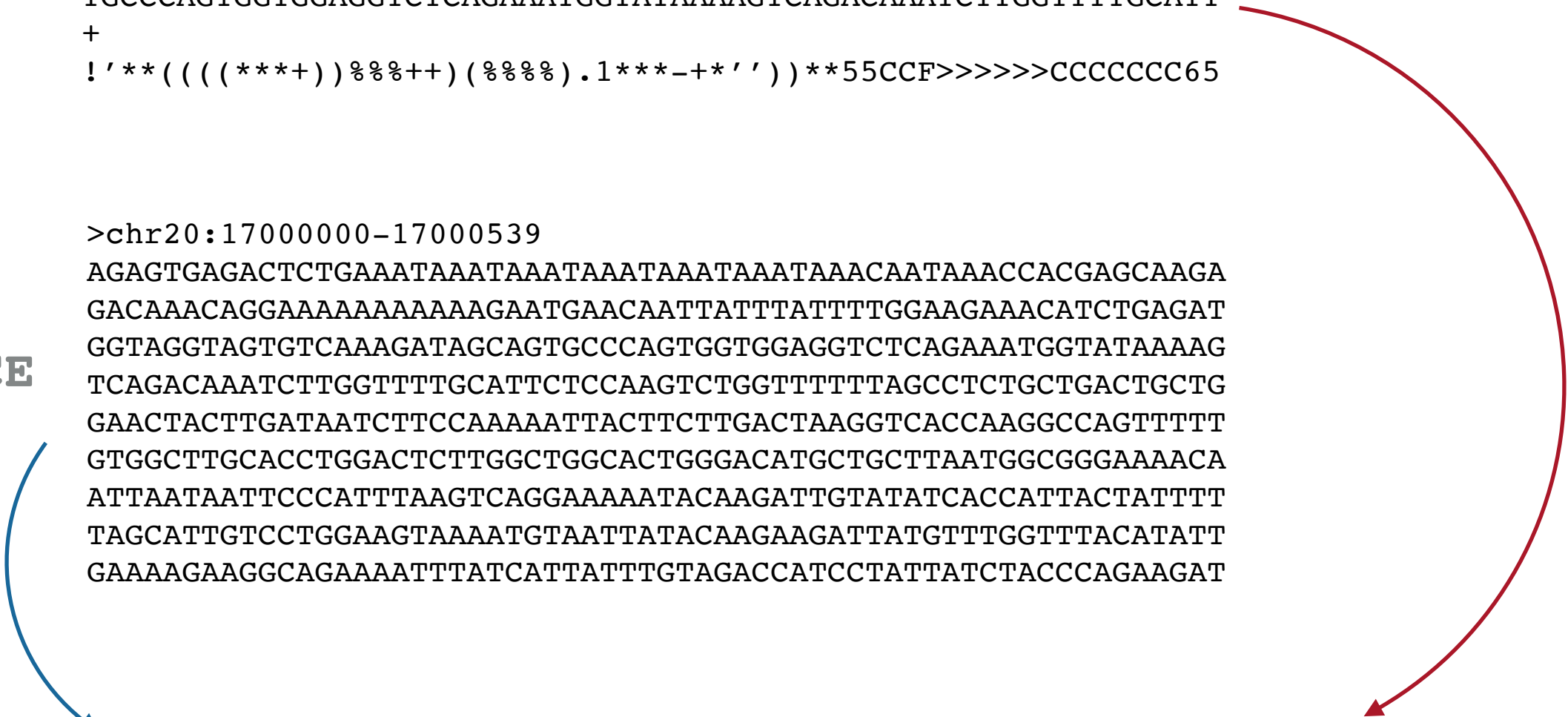
```
@SEQ_ID
TGCCCAGTGGTGGAGGTCTCAGAAATGGTATAAAAGTCAGACAAATCTTGGTTTTGCATT
+
!'**((( (***) )%%%++) (%%%) .1***-+*' ' ) **55CCF>>>>>CCCCCCC65
```

REFERENCE

```
>chr20:17000000-17000539
AGAGTGAGACTCTGAAATAAATAAATAAATAAATAAATAAACAATAAACCACGAGCAAGA
GACAAACAGGAAAAAAAAAAGAATGAACAATTATTTATTTTGGGAAGAAACATCTGAGAT
GGTAGGTAGTGTCAAAGATAGCAGTGCCCAGTGGTGGAGGTCTCAGAAATGGTATAAAAG
TCAGACAAATCTTGGTTTTGCATTCTCCAAGTCTGGTTTTTTAGCCTCTGCTGACTGCTG
GAACTACTTGATAATCTTCCAAAAATTACTTCTTGACTAAGGTCACCAAGGCCAGTTTTT
GTGGCTTGCACCTGGACTCTTGGCTGGCACTGGGACATGCTGCTTAATGGCGGGAAAACA
ATTAATAATTCCCATTTAAGTCAGGAAAAATACAAGATTGTATATCACCATTACTATTTT
TAGCATTGTCCTGGAAGTAAAATGTAATTATACAAGAAGATTATGTTTGGTTTACATATT
GAAAAGAAGGCAGAAAATTTATCATTATTTGTAGACCATCCTATTATCTACCCAGAAGAT
```

ALIGNMENT

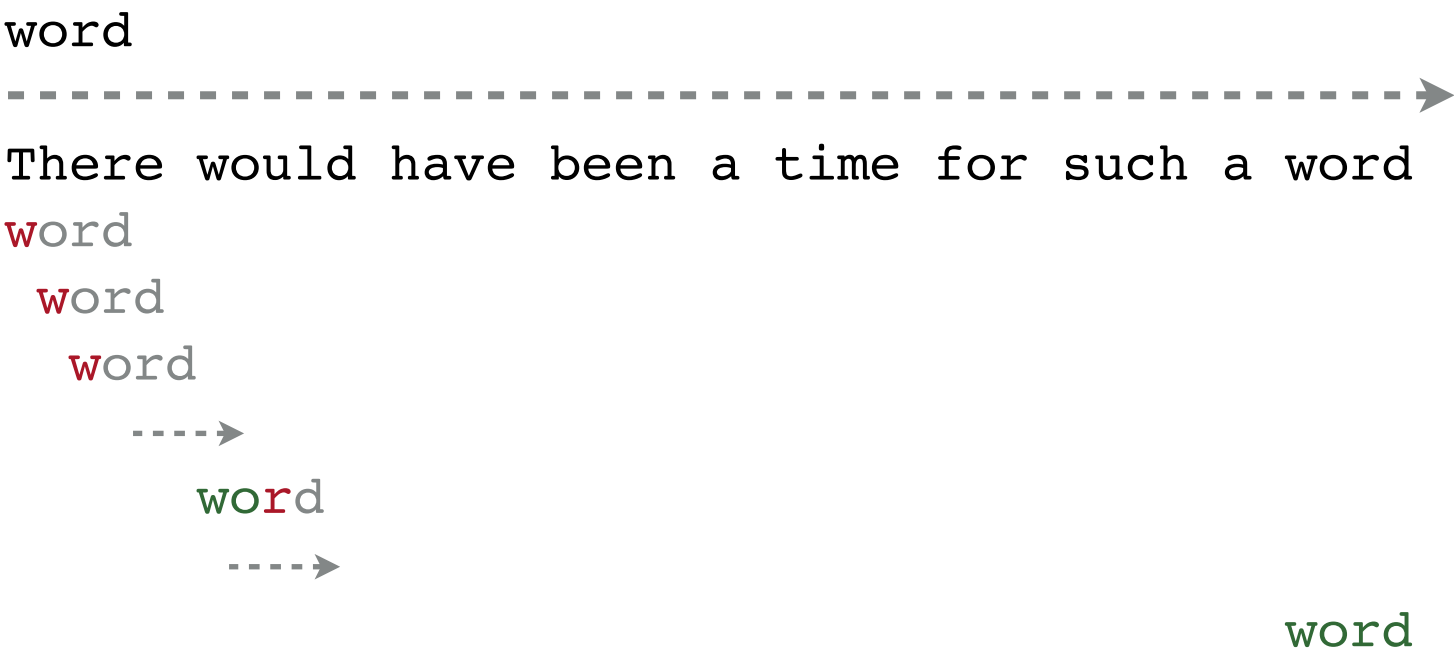
```
TGCCCAGTGGTGGAGGTCTCAGAAATGGTATAAAAGTCAGACAAATCTTGGTTTTGCATT
TAGCAGTGCCCAGTGGTGGAGGTCTCAGAAATGGTATAAAAGTCAGACAAATCTTGGTTTTGCATTCTCCAA
```



2. EXACT STRING MATCHING ALGORITHMS

P: word

T: There would have been a time for such a word



There would have been a time for such a word

word word word word word word word word word word

word word word word word word word word word

word word word word word word word word word

word word word word word word word word word

word word word word word word word word word

Q: How many alignments ?

A: $|T| - |P| + 1$

Q: How many character comparison ?

A: Range from $|T| - |P| + 1$ (best case) to $|P| * (|T| - |P| + 1)$ (worst case)

Q: How this can be improved ?

A: Learn from character comparisons to skip pointless alignments

word

----->

There would have been a time for such a word

word

----> skip

----> skip

word

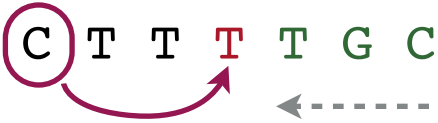
#As 'u' not in 'word', skip the next 2 alignments

Boyer Moore's rules:

1. Test alignments from left to right, but perform character comparisons in opposite direction
2. Upon hitting a mismatch, skip alignments until mismatch becomes a match or P moves past the mismatch (aka, BAD CHARACTER RULE)
3. Let t be a substring of T matched by a substring of P. Upon hitting a mismatch, skip alignments until there are no mismatches between P and t/a prefix of P matches a suffix of T or P moves past t (aka, GOOD CHARACTER RULE)


T: G C T T C T G C T A C C T T T T G C G C G C G C G C G G A A

P: C C T T T T G C



T: G C T T C T G C T A C C T T T T G C G C G C G C G C G G A A

P: 2 skips C C T T T T G C



T: G C T T C T G C T A C C T T T T G C G C G C G C G C G G A A

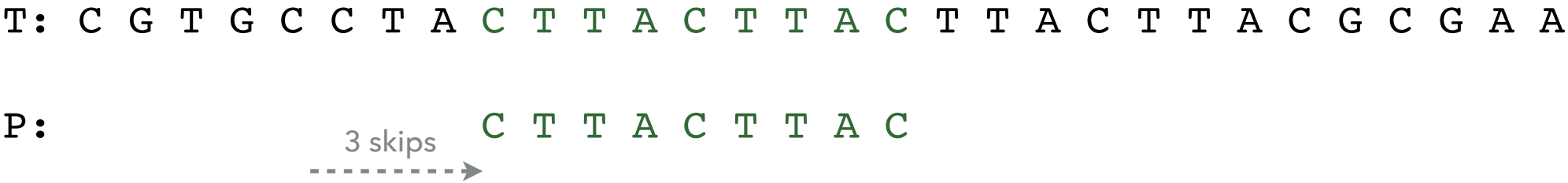
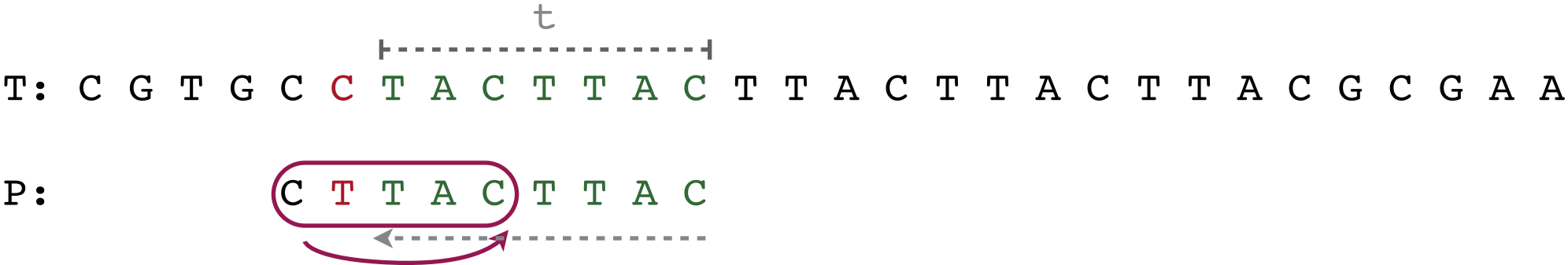
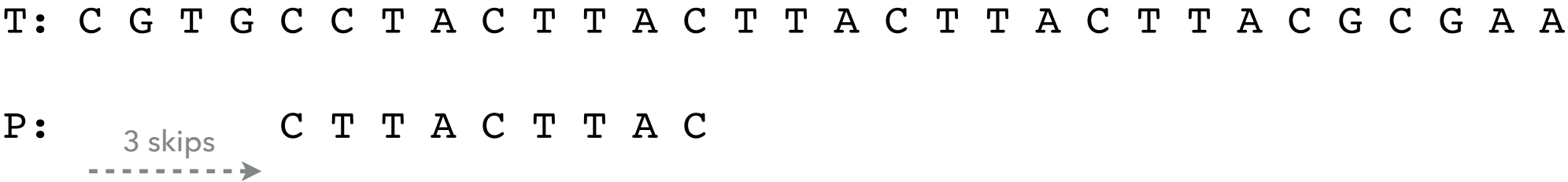
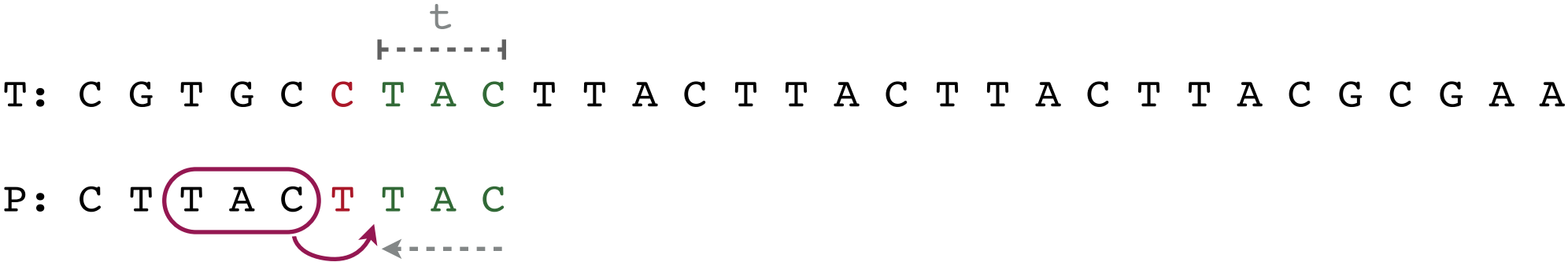
P: C C T T T T G C



T: G C T T C T G C T A C C T T T T G C G C G C G C G C G G A A

P: C C T T T T G C





T: G T T A T A G C T G A T C G C G G C G T A G C G G C G A A

P: G T A G C G G C G

Bad character rule: skip 6
Good character rule: N.A

T: G T T A T A G C T G A T C G C G G C G T A G C G G C G A A

P: G T A G C G G C G

Bad character rule: skip 0
Good character rule: skip 2

T: G T T A T A G C T G A T C G C G G C G T A G C G G C G A A

P: G T A G C G G C G

Bad character rule: skip 2
Good character rule: skip 7

T: G T T A T A G C T G A T C G C G G C G T A G C G G C G A A

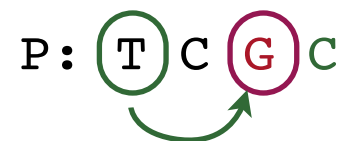
P: G T A G C G G C G

		P			
		T	C	G	C
Σ	A	0	1	2	3
	C	0	–	0	–
	G	0	1	–	0
	T	–	0	1	2

Bad character rule: lookup table for P

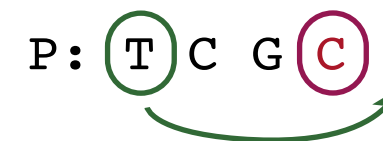
T: A A T C A A T A G C

P: T C G C



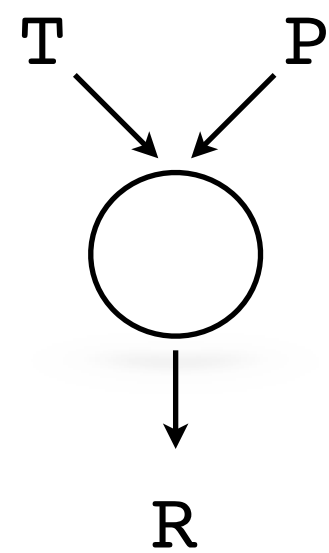
T: C G C A T G C G A G

P: T C G C



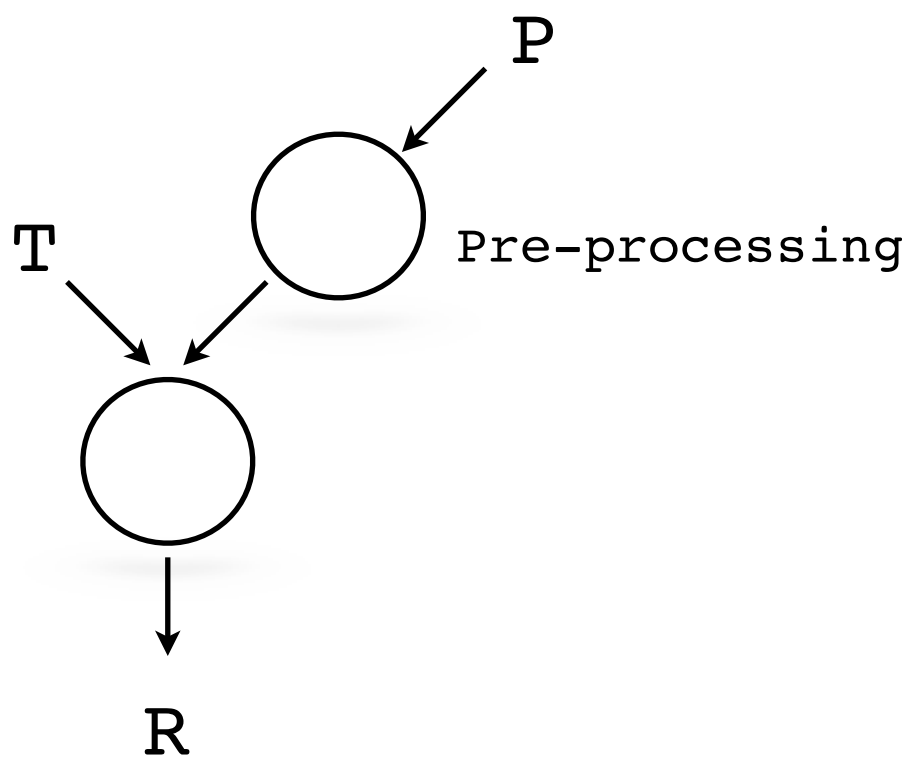
Q: Any benefits?

A: Overall work reduced via reuse

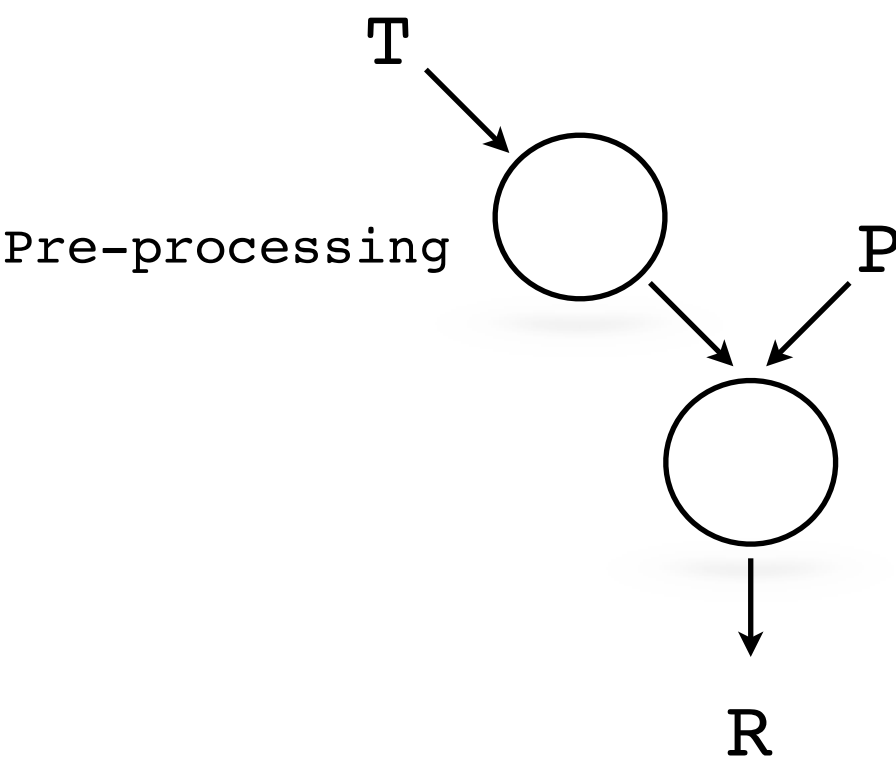


Brute Force String Matching

ONLINE

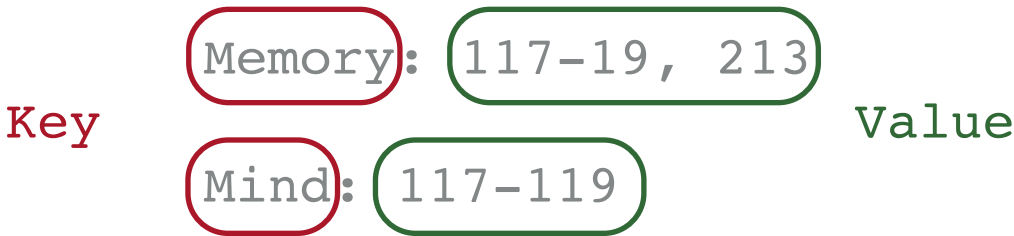


Boyer-Moore String Matching

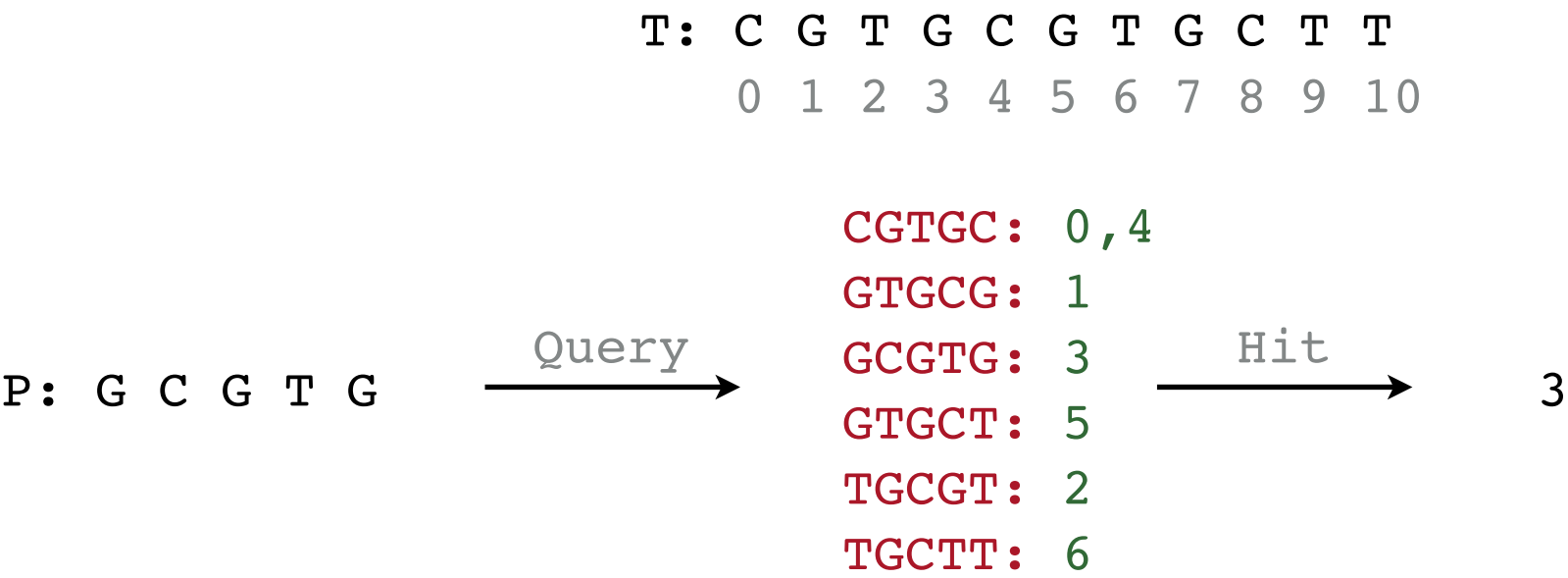


OFFLINE

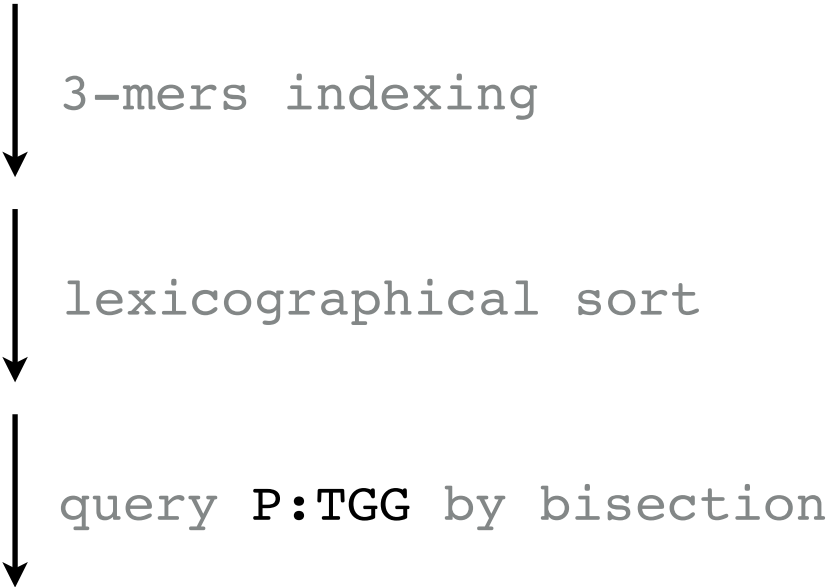
Index of a book for terms 'memory' and 'mind'



Index of DNA string T for 5-mers substrings



T: G T G C G T G T G G G G G



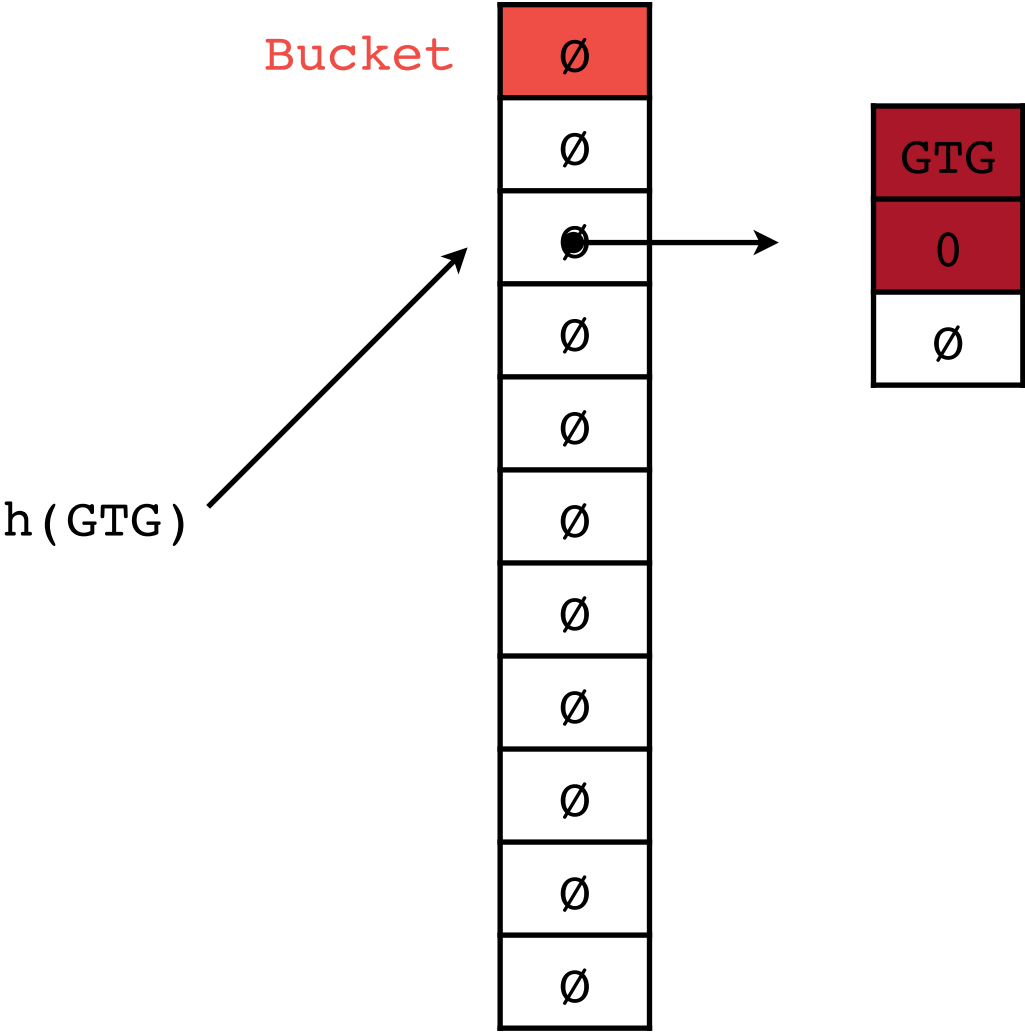
CGT	3
GCG	2
GGG	8,9,10
GTG	0,4,6
TGC	1
TGG	7
TGT	5

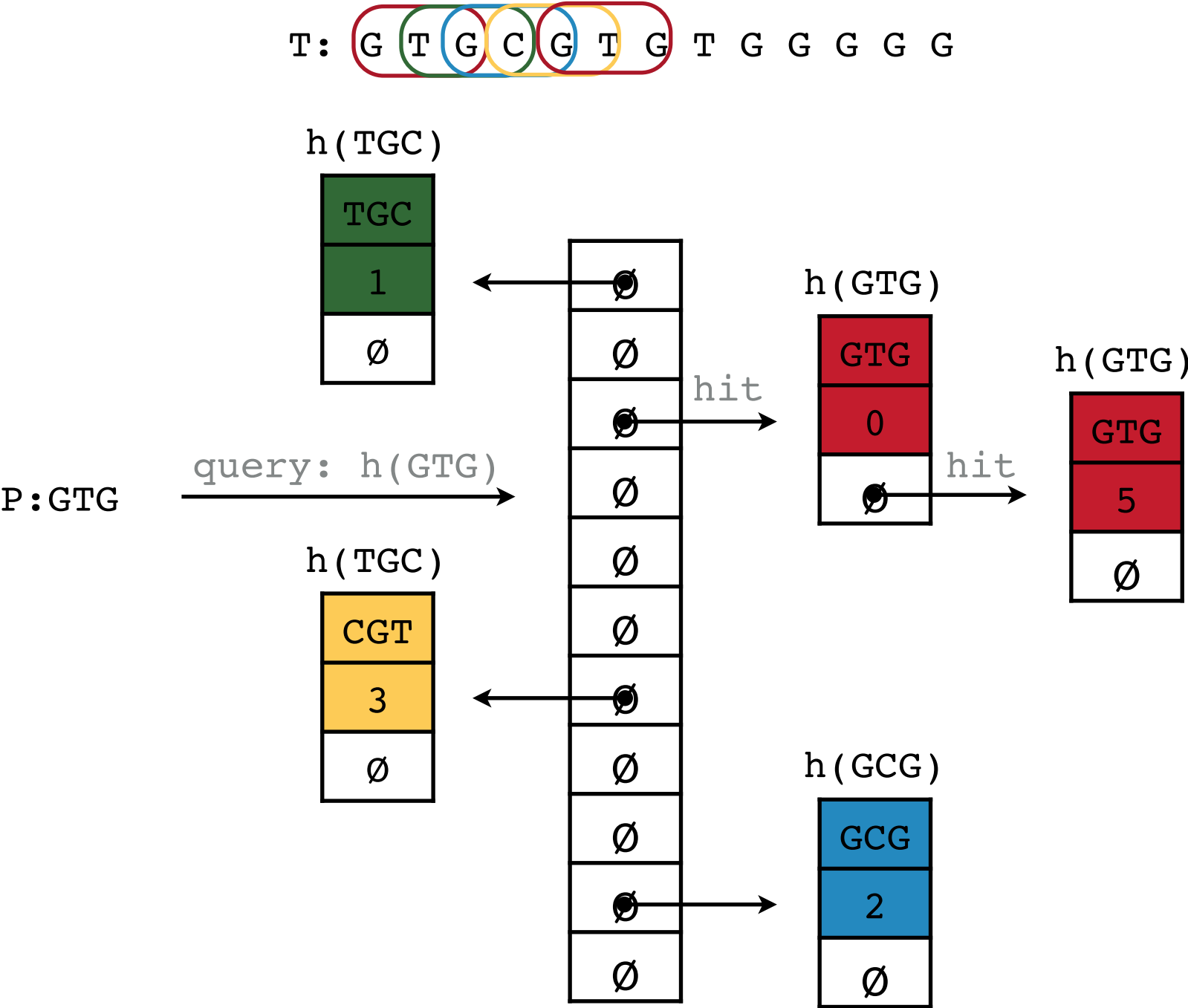
TGG > GTG
 query

CGT	3
GCG	2
GGG	8,9,10
GTG	0,4,6
TGC	1
TGG	7
TGT	5

TGG = TGG
 hit

T: G T G C G T G T G G G G G
0 1 2 3 4 5 ...





3. A BIOINFORMATIC EXAMPLE

```
In [1]: !wget --no-check https://d28rh4a8wq0iu5.cloudfront.net/ads1/data/phix.fa

--2019-10-16 16:17:36-- https://d28rh4a8wq0iu5.cloudfront.net/ads1/data/phix.fa
Risoluzione di d28rh4a8wq0iu5.cloudfront.net (d28rh4a8wq0iu5.cloudfront.net)... 143.204.10.105, 143.204.10.216, 143.204.10.21, ...
Connessione a d28rh4a8wq0iu5.cloudfront.net (d28rh4a8wq0iu5.cloudfront.net)|143.204.10.105|:443... connesso.
Richiesta HTTP inviata, in attesa di risposta... 200 OK
Lunghezza: 5528 (5,4K) [application/octet-stream]
Salvataggio in: "phix.fa"

phix.fa          100%[=====>]    5,40K  --.-KB/s    in 0s

2019-10-16 16:17:37 (195 MB/s) - "phix.fa" salvato [5528/5528]
```

```
In [2]: def ReadGenome(filename):
        '''
        Open and read a FASTA, concatenating different sequences into a single one
        '''
        genome=''
        with open(filename, 'r') as f:
            for line in f:
                if line[0] != '>':
                    genome+=line.rstrip()
        return genome
```

```
In [3]: genome=ReadGenome('phix.fa')
```

```
In [4]: len(genome)
```

```
Out[4]: 5386
```

```
In [1]: !wget --no-check https://d28rh4a8wq0iu5.cloudfront.net/ads1/data/ERR266411_1.first1000.fastq

--2019-10-16 16:31:07-- https://d28rh4a8wq0iu5.cloudfront.net/ads1/data/ERR266411_1.first1000.fastq
Risoluzione di d28rh4a8wq0iu5.cloudfront.net (d28rh4a8wq0iu5.cloudfront.net)... 143.204.10.21, 143.204.10.216, 143.204.10.34, ...
Connessione a d28rh4a8wq0iu5.cloudfront.net (d28rh4a8wq0iu5.cloudfront.net)|143.204.10.21|:443... connesso.
Richiesta HTTP inviata, in attesa di risposta... 200 OK
Lunghezza: 254384 (248K) [audio/mpeg]
Salvataggio in: "ERR266411_1.first1000.fastq"

ERR266411_1.first10 100%[=====>] 248,42K  777KB/s   in 0,3s

2019-10-16 16:31:08 (777 KB/s) - "ERR266411_1.first1000.fastq" salvato [254384/254384]
```

```
In [2]: def ReadFastq(filename):

        '''
        Open and read a 4-line FASTQ. Discard first/third/fourth line for each sequence
        '''

        sequences=[]
        with open(filename, 'r') as f:
            while True:
                f.readline()
                seq=f.readline().rstrip()
                if seq == '':
                    break
                sequences.append(seq)
                f.readline()
                f.readline()

        return sequences
```

```
In [3]: reads=ReadFastq('ERR266411_1.first1000.fastq')
```

```
In [4]: len(reads)
```

```
Out[4]: 1000
```

```
In [6]: len(reads[0])
```

```
Out[6]: 100
```

```
In [10]: def BruteForce(P, T):
        '''
        Simple implementation of Brute Force String Matching
        '''

        assert len(P) <= len(T)
        occurrences = []
        for i in range(len(T)-len(P)+1):
            match = True
            for j in range(len(P)):
                if T[i+j] != P[j]:
                    match = False
                    break
            if match:
                occurrences.append(i)
        return occurrences

In [11]: total=0
        matched=0

        for read in reads:
            matches=BruteForce(read,genome)
            if len(matches) > 0:
                matched += 1
            total+=1

        print('%d / %d reads matched the genome !' %(matched,total))
```

7 / 1000 reads matched the genome !

Q: Why this number is so small?

A1: Differences between individuals and sequenced reads

A2: Reads orientation

```
In [13]: total=0
         matched=0
         trans = str.maketrans('AaTtGgCcN', 'TtAaCcGgN')

         for read in reads:
             matches=BruteForce(read,genome)
             if len(matches) > 0:
                 matched += 1
             else:
                 revMatches=BruteForce(read[::-1].translate(trans), genome)
                 if len(revMatches) > 0:
                     matched +=1
             total+=1

         print('%d / %d reads matched the genome !' %(matched,total))
```

449 / 1000 reads matched the genome !

Q: Why this number is (still) so small?

A3: Sequencing errors

```
In [14]: total=0
matched=0
trans = str.maketrans('AaTtGgCcN', 'TtAaCcGgN')

for read in reads:
    read=read[:30]
    matches=BruteForce(read,genome)
    if len(matches) > 0:
        matched += 1
    else:
        revMatches=BruteForce(read[::-1].translate(trans), genome)
        if len(revMatches) > 0:
            matched +=1
    total+=1

print('%d / %d reads matched the genome !' %(matched,total))
```

932 / 1000 reads matched the genome !

Take-home message #2

Sequencing errors occur (% is ~1% in short reads and ~10% in long reads) and exact string matching algorithms are not the method of choice for mapping

4. APPROXIMATE STRING MATCHING ALGORITHMS

Mismatch

T: G G A A A A A G A G G T A G C G G C G T T T A A C A G T A G

P: G T A A C G G C G

Insertion

T: G G A A A A A G A G G T A G C - G C G T T T A A C A G T A G

P: G T A G C G G C G

Deletion

T: G G A A A A A G A G G T A G C G G C G T T T A A C A G T A G

P: G T - G C G G C G

Given T and P, with $|T| = |P|$, the Hamming Distance is the minimum #substitutions required to turn one into the other

T: G G A A A A A G A G G T A G C G G C G T T T A A C A G T A G

P: G G T T A A A G A G G G A G C G G C G T T T T A C A G T A G

Hamming distance = 4

Given T and P, the Edit Distance (aka, Levenshtein Distance) is the minimum #edits (substitutions, insertions or deletions) required to turn one into the other

T: G G A A A A A G A G G T A G C G G C G T T T A A C A G T A G

P: G G T A A A A G A G G T A G C G G C G T T T A C A G T A G

Edit distance = 2

Q: What is the relationship between Hamming and Edit Distance if $|T| = |P|$?

T: G C G T A T G C G G C T A A C G C

P: G C T A T G C G G C T A T A C G C



T: G C G T A T G C G G C T A A C G C

P: G C T A T G C G G C T A T A C G C

Hamming distance = 10

T: G C G T A T G C G G C T A - A C G C

P: G C - T A T G C G G C T A T A C G C

Edit distance = 2

A: Edit Distance (P,T) \leq Hamming Distance (P,T)

Q: What is the minimum Edit Distance if $|P| \neq |T|$?

A: Edit Distance (P,T) $\geq ||T| - |P||$

```
T:  G C T A T A C
    0 1 2 3 4 5 6
P:  G C G T A T G C
    0 1 2 3 4 5 6 7
```

Diagram illustrating a dynamic programming table for sequence alignment. The table is labeled with sequence lengths $|P| + 1$ and $|T| + 1$. The table shows the alignment of the sequence $GATTACA$ (P) against the sequence $GATTACA$ (T). The cell at (1,1) is highlighted with a blue border and a blue circle, indicating the current state. A black arrow points from the bottom-right towards this cell.

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

Table D

```
D[i,j] = min(
    D[i-1,j]+1,
    D[i,j-1]+1,
    D[i-1,j-1]+a,
)
```

$$\partial = 1 \text{ if } T[i-1] \neq P[j-1] \text{ else } 0$$

N.B: i are indexes for T
 j are indexes for P

```
D[1,1] = min(
    1+1,
    1+1,
    0+0,
) = 0
```

T: G C T A T A C

P: G C G T A T G C

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



Edit Distance between T and P

T: T A T T G G C T A T A C G G T T

T: T A T T G G C - T A T A C G G T T

P: G C G T A T G C

P: G C G T A T G C

Apply the Edit Distance formula

Initialize first row with 0s

	ε	T	A	T	T	G	G	C	T	A	T	A	C	G	G	T	T
ε	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M G	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	1	1
M C	2	2	2	2	2	1	1	0	1	2	2	2	1	1	1	1	2
I G	3	3	3	3	3	2	1	1	1	2	3	3	2	1	1	2	2
M T	4	3	4	3	3	3	2	2	1	2	2	3	3	2	2	1	2
M A	5	4	3	4	4	4	3	3	2	1	2	2	3	3	3	2	2
M T	6	5	4	3	4	5	4	4	3	2	1	2	3	4	4	3	2
S G	7	6	5	4	4	4	5	5	4	3	2	2	3	3	4	4	3
M C	8	7	6	5	5	5	5	5	5	4	3	3	2	3	4	5	4

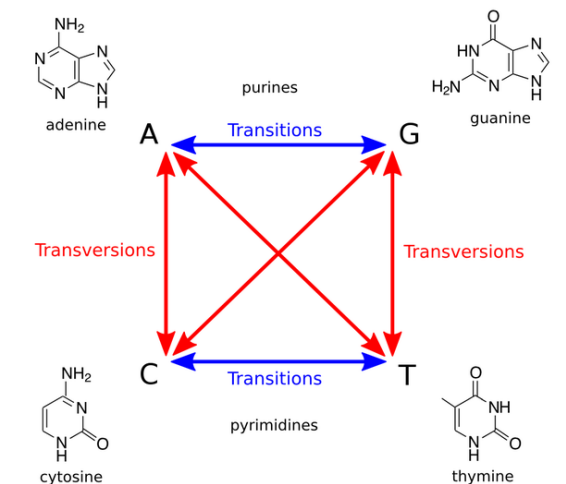
Trace back the matrix using the minimum parental value

Find the minimum value in the last row

Edit Distance-based approximate string matching penalize different kind of edits we may find with the same amount: that is, there is no difference between a substitution and a deletion or different kind of substitutions

Q: Is this correct?

	AFR		AMR		EAS		EUR		SAS	
Samples	661		347		504		503		489	
Mean coverage	8.2		7.6		7.7		7.4		8.0	
	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons	Var. sites	Singletons
SNPs	4.31M	14.5k	3.64M	12.0k	3.55M	14.8k	3.53M	11.4k	3.60M	14.4k
Indels	625k	-	557k	-	546k	-	546k	-	556k	-
Large deletions	1.1k	5	949	5	940	7	939	5	947	5
CNVs	170	1	153	1	158	1	157	1	165	1
MEI (Alu)	1.03k	0	845	0	899	1	919	0	889	0
MEI (L1)	138	0	118	0	130	0	123	0	123	0
MEI (SVA)	52	0	44	0	56	0	53	0	44	0
MEI (MT)	5	0	5	0	4	0	4	0	4	0
Inversions	12	0	9	0	10	0	9	0	11	0



Transition/Transversion ~2.1

A global reference for human genetic variation

A: No. We need something similar to the Edit Distance-based approximate string matching but with personalized penalties (that is, give an higher penalty to deletions rather than to substitutions or an higher penalty to transversions rather than to transitions). Global Alignment (aka, Needleman-Wunsch algorithm) and Local Alignment (aka, Smith-Waterman algorithm) serve the purpose

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

Penalties:

- 0 for matches
- 8 for gaps
- 2 for transitions
- 4 for transversions

T: T A T G T C A T G C

0 1 2 3 4 5 6 7 8 9

P: T A C G T C A G G

0 1 2 3 4 5 6 7 8

Initialize first row and first column with gap penalties

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

Table D

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

Apply the formula:

$$D[i,j] = \min(\begin{aligned} &D[i-1,j]+p(T[i-1], '-'), \\ &D[i,j-1]+p('-', P[j-1]), \\ &D[i-1,j-1]+p(T[i-1], P[j-1]), \\ & \end{aligned})$$

N.B i are indexes of T
j are indexes of P

$$\begin{aligned} D[1,1] = \min(\\ &8+8, \\ &8+8, \\ &0+0, \\ &) = 0 \end{aligned}$$

T: T A T G T C A T G C

T: T A T G T C A T G C

P: T A C G T C A G C

P: T A C G T C A - G C

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

		€	T	A	T	G	T	C	A	T	G	C
	€	0	8	1 6	2 4	3 2	4 0	4 8	5 6	6 4	7 2	8 0
M	T	8	0	8	1 6	2 4	3 2	4 0	4 8	5 6	6 4	7 2
M	A	1 6	8	0	8	1 6	2 4	3 2	4 0	4 8	5 6	6 4
S	C	2 4	1 6	8	2	1 0	1 8	2 4	3 2	4 0	4 8	5 6
M	G	3 2	2 4	1 6	1 0	2	1 0	1 8	2 6	3 4	4 0	4 8
M	T	4 0	3 2	2 4	1 6	1 0	2	1 0	1 8	2 6	3 4	4 2
M	C	4 8	4 0	3 2	2 4	1 8	1 0	2	1 0	1 8	2 6	3 4
M	A	5 6	4 8	4 0	3 2	2 6	1 8	1 0	2	1 0	1 8	2 6
DM	G	6 4	5 6	4 8	4 0	3 2	2 6	1 8	1 0	6	1 0	1 8
M	C	7 2	6 4	5 6	4 8	4 0	3 4	2 6	1 8	1 2	1 0	1 0



Start to trace back from bottom rightmost cell

Given 2 strings (T and P), Local Alignment does not try to find the alignment of P to T but try to identify the substring of T and the substring of P which are the most similar to each other

T: he will after his sour fashion tell you

P: struts and frets his hour upon the stage

T: he will after **his sour** fashion tell you

P: struts and frets **his hour** upon the stage

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

Rewards:
2 for matches
-6 for gaps
-4 for substitutions

T: T A T A T G C G G C G T T T
0 1 2 3 4 5 6 7 8 9 ...
P: G G T A T G C T G G C G C T A
0 1 2 3 4 5 6 7 8 9 ...

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

Initialize first row and first column with 0s

	ε	T	A	T	A	T	G	C	G	G	C	G	T	T	T
ε	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0														
G	0														
T	0														
A	0														
T	0														
G	0														
C	0														
T	0														
G	0														
C	0														
G	0														
C	0														
T	0														
A	0														

Table D

Apply the formula:

$$D[i,j] = \max(\begin{aligned} &D[i-1,j]+r(T[i-1], '-'), \\ &D[i,j-1]+r('-', P[j-1]), \\ &D[i-1,j-1]+r(T[i-1], P[j-1]), \\ &0 \end{aligned})$$

N.B i are indexes of T
j are indexes of P

$$D[1,1] = \min(\begin{aligned} &0+(-6), \\ &0+(-6), \\ &0+(-4), \\ &0 \end{aligned}) = 0$$

T: T A T A T G C G G C G T T T T: T A T A T G C - G G C G T T T

P: G G T A T G C T G G C G C T A P: G G T A T G C T G G C G C T A

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

Stop to trace back when reach 0

	ε	T	A	T	A	T	G	C	G	G	C	G	T	T	T
ε	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	2	0	2	2	0	2	0	0	0
G	0	0	0	0	0	0	2	0	2	4	0	2	0	0	0
M T	0	2	0	2	0	2	0	0	0	0	0	0	4	2	2
M A	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0
M T	0	2	0	6	0	6	0	0	0	0	0	0	2	2	2
M G	0	0	0	0	2	0	8	2	2	2	0	2	0	0	0
M C	0	0	0	0	0	0	2	1 0	4	0	4	0	0	0	0
I T	0	2	0	2	0	2	0	4	6	0	0	0	2	2	2
M G	0	0	0	0	0	0	4	0	6	8	2	2	0	0	0
M G	0	0	0	0	0	0	2	0	2	8	4	4	0	0	0
M C	0	0	0	0	0	0	0	4	0	2	1 0	4	0	0	0
M G	0	0	0	0	0	0	2	0	6	2	4	1 2	6	0	0
C	0	0	0	0	0	0	0	4	0	2	4	6	8	2	0
T	0	2	0	2	0	2	0	0	0	0	0	0	8	1 0	4
A	0	0	4	0	4	0	0	0	0	0	0	0	2	4	6

Start to trace back from cell with the highest value

Q: Is there a unique way of performing Global and Local alignments ?

A: No. It depends on the values we use in penalty and score matrixes. Moreover, usually there is a different score for gap-opening and gap-extending (the cost for gap-extending is usually lower)

Q: What is the time complexity of dynamic programming ?

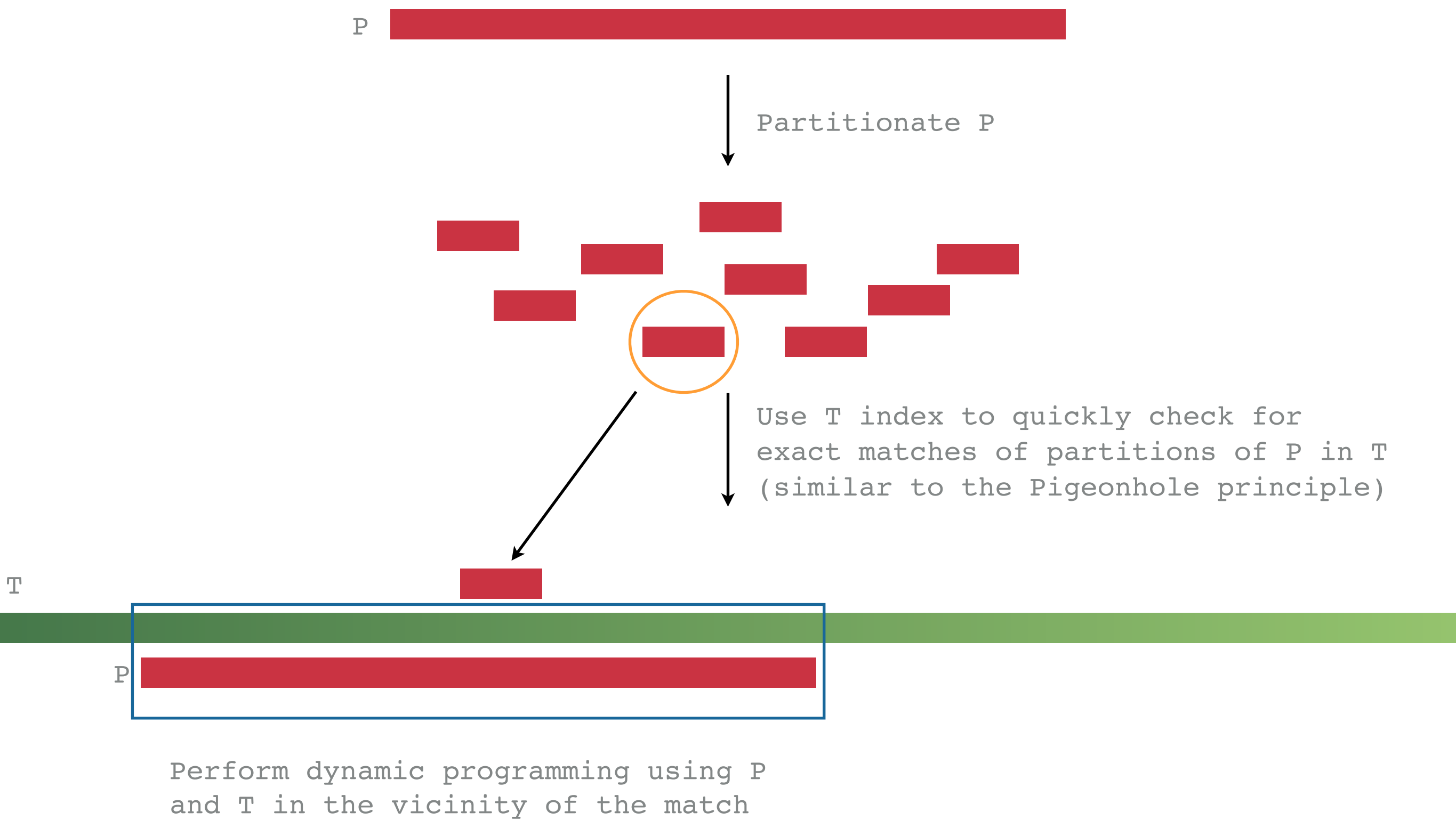
A: $O(mn)$

Q: What is the space complexity of dynamic programming ?

A: $O(mn)$

Q: Can Global and Local alignments algorithms be applied as described here ?

A: No. The human reference genome $\approx 3 \times 10^9$ nucleotides (m). 1-week long run of an Illumina HiSeq 4000 produce $\approx 6 \times 10^9$ reads (d), 100 nucleotides each (n). Time (and space, even if we can reduce this somehow) are then $O(mnd)$. With 1000 processors, 3 GHz each, this will require ≈ 2 years



Take-home message #3

Index-assisted global and local alignment algorithms combine dynamic programming's efficacy (that is, they produce the results we want) and indexes' efficiency (that is, they do not waste too much time doing what they do) and are the method of choice for mapping the reads to the genome

5. A TRUE TO LIFE EXAMPLE

nature|methods

Brief Communication | Published: 04 March 2012

Fast gapped-read alignment with Bowtie 2

Ben Langmead  & Steven L Salzberg

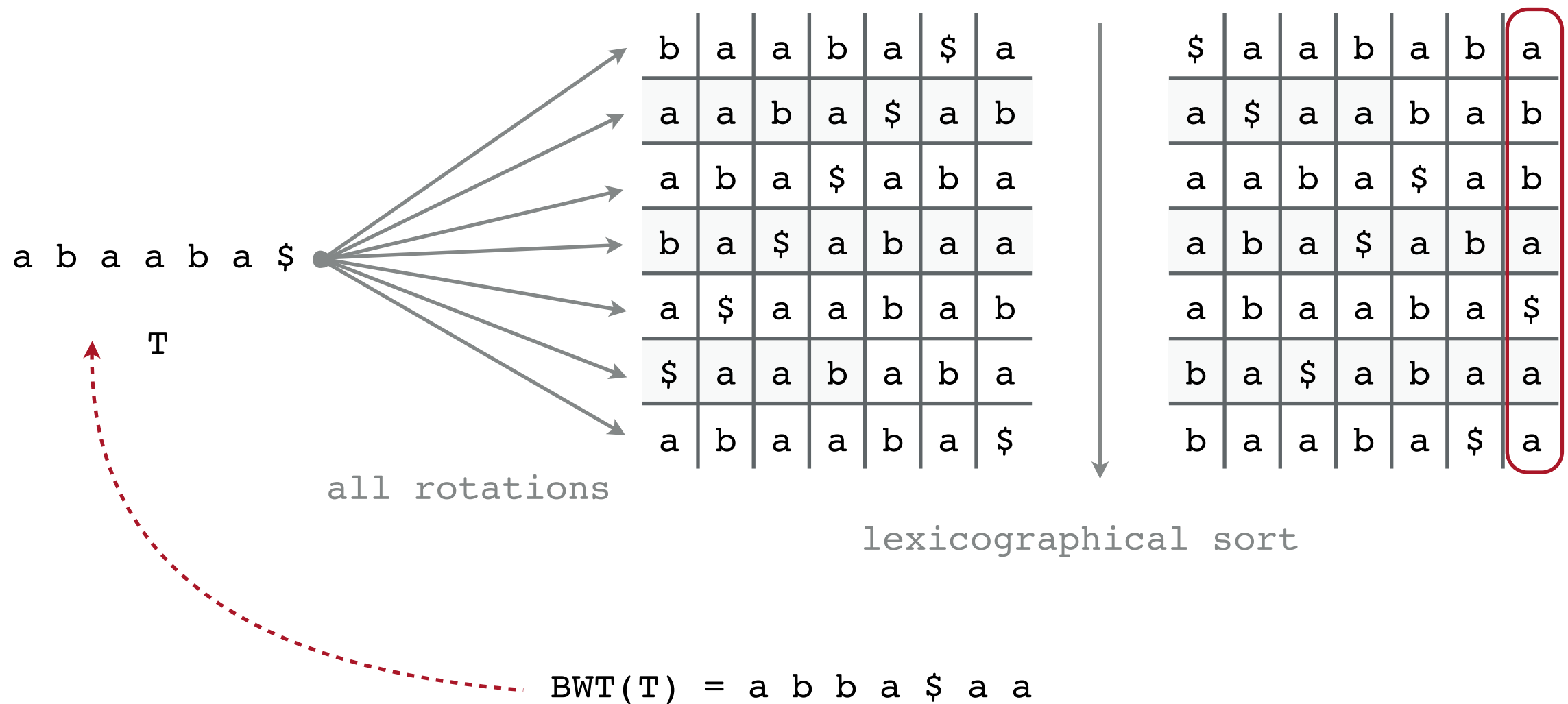
Nature Methods **9**, 357–359 (2012) | [Download Citation](#) ↓

9132 Accesses | **11490** Citations | **75** Altmetric | [Metrics](#) >>

Abstract

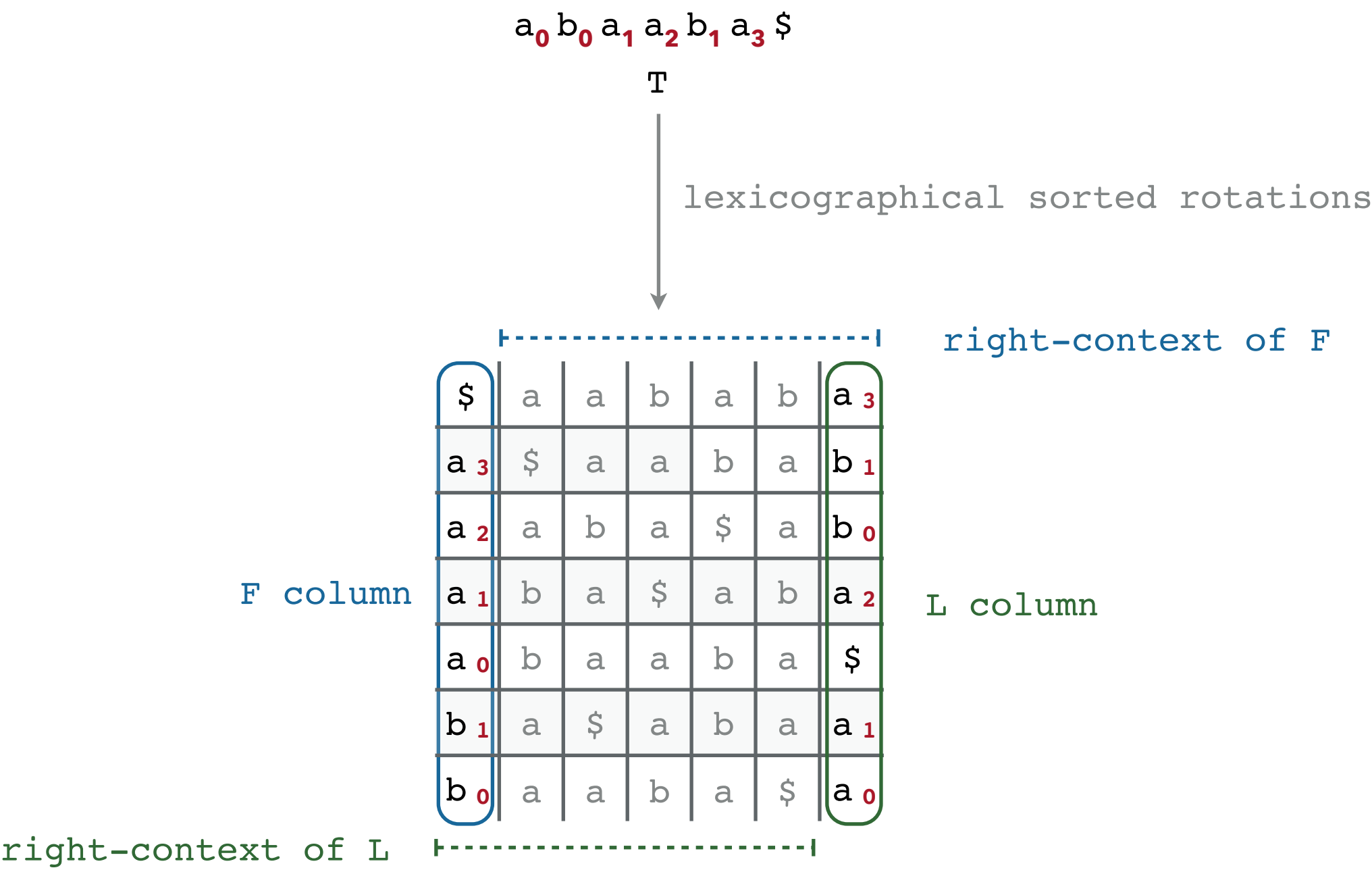
As the rate of sequencing increases, greater throughput is demanded from read aligners. The full-text minute index is often used to make alignment very fast and memory-efficient, but the approach is ill-suited to finding longer, gapped alignments. Bowtie 2 combines the strengths of the full-text minute index with the flexibility and speed of hardware-accelerated dynamic programming algorithms to achieve a combination of high speed, sensitivity and accuracy.

The Burrows-Wheeler transform (BWT) of a string T is the reversible permutation of all its characters, originally used for compression (for example, it is the core of bzip2)

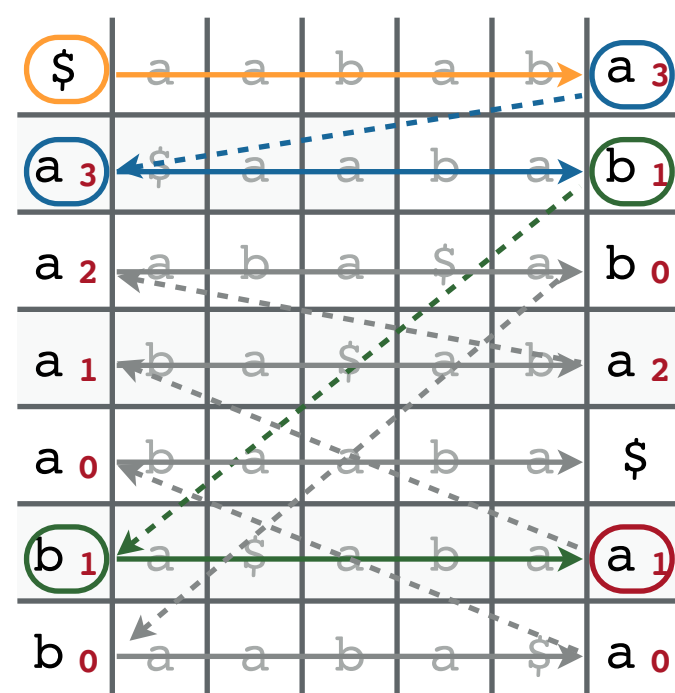


Q: How do we revert this ?

Give each character in T a rank, equal to the #times the character occurred previously in T



The i^{th} occurrence of a character c in L and the i^{th} occurrence of c in F have the same rank (that is, correspond to same occurrence in T). This is because they are sorted by their right-context



Start in F, first row, from \$

L contains character prior to \$: a₃

Move to a₃ in F

L contains character prior to a₃: b₁

Move to b₁ in F

L contains character prior to b₁: a₁

Continue until reversion is complete

a b a a b a \$ = T

We proceed by trying to match longer suffixes of P to BWT(T)

P: a b a

	\$	a	a	b	a	b	a ₃
	a ₃	\$	a	a	b	a	b ₁
	a ₂	a	b	a	\$	a	b ₀
	a ₁	b	a	\$	a	b	a ₂
	a ₀	b	a	a	b	a	\$
	b ₁	a	\$	a	b	a	a ₁
	b ₀	a	a	b	a	\$	a ₀

Get the range of rows beginning with a
Now seek rows starting with ba
Look at the same occurrences in F
Seek rows starting with aba
Look at the same occurrences in F

Get the range of the BWT(T) matching P

a ₁	b	a	\$	a	b	a ₂
a ₀	b	a	a	b	a	\$

We won't talk about why the FM index method is fast and how all these informations are stored in a limited space. We need just to sort out one last thing...

T: a b a a b a
0 1 2 3 4 5

P: a b a

\$	a	a	b	a	b	a
a	\$	a	a	b	a	b
a	a	b	a	\$	a	b
a	b	a	\$	a	b	a
a	b	a	a	b	a	\$
b	a	\$	a	b	a	a
b	a	a	b	a	\$	a

BWT (T)

≈

6	\$	a	a	b	a	b	a
5	a	\$	a	a	b	a	b
2	a	a	b	a	\$	a	b
3	a	b	a	\$	a	b	a
0	a	b	a	a	b	a	\$
4	b	a	\$	a	b	a	a
1	b	a	a	b	a	\$	a

SA (T)

Suffix arrays store every possible suffix of T and its offset but this requires a lot of space (~12 Gb for the human genome), while an FM index is small (just ~1.5 Gb for the same reference)

T: a b a a b a
0 1 2 3 4 5

P: a b a

	\$	a	a	b	a	b	a ₃	6	
	a ₃	\$	a	a	b	a	b ₁		
	a ₂	a	b	a	\$	a	b ₀	2	✓
	a ₁	b	a	\$	a	b	a ₂		✗
	a ₀	b	a	a	b	a	\$	0	✓
	b ₁	a	\$	a	b	a	a ₁	4	
	b ₀	a	a	b	a	\$	a ₀		

BWT (T)

Check if index lookup succeed
If it succeeds, keep the index
If not, apply LF mapping
If it succeeds, keep the index
Continue until all the indexes are found

We got 1 hit at index 0 and one at index 2 + 1

6. TEACHING MATERIAL

1. Email me @:
davide.bolognini@unifi.it
2. Get slides (.key + .pdf) from GitHub:
<https://github.com/davidebolo1993/Classes>

THAT ' ALL , FOLKS !
