Alignment of short/long-read sequencing data

2019 Dragon Star Bioinformatics Course (Day 2)

Sequence similarity

- For any two sequences
  - Mutation: mismatch
  - Insertion
  - Deletion

DNA-sequence-1

```
tcctctgcctcgatgccatcat--caaccacaagt
|||| ||| ||  ||| |||||  ||||||| ||||
tcctgtgcatc--tgcaatcatggcaaccagaagt
```

DNA-sequence-2

Sequence alignment

- Sequence alignment
  - Consider matches, mismatches, insertions and deletions (indels are gaps)
  - Find an optimal alignment between sequences

  - Applications
    - Reference-based read mapping
    - Genome assembly
    - Gene finding

Pairwise alignment

- Sequence alignment between two sequences
  - Find an optimal alignment between two sequences
  - Dynamic programming
    - Input
      - Two sequences
      - Score matrix

Dynamic programming - score matrix

- Simple scoring matrix
  - *Assume: a, b are two bases*
  - $$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -2 \; if \; b = gap \end{cases}$$ *or* $$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -2 \; for \; open \; gaps \\ -1 \; for \; extended \; gaps \end{cases}$$

	A	C	G	T
A	2	-3	-3	-1
C	-2	3	-1	-2
G	-2	-1	4	-3
T	-1	-1	-2	1

Dynamic programming

- Purpose:
  - To find an alignment between two sequences with best matching scores
- Three components
  - Recursive calculation
  - Tabular arrangement
  - Traceback
- Three common types of pairwise alignments
  - Global alignment: Needleman-Wunsch
  - Local alignment: Smith-Waterman
  - Semi-global alignment

Global alignment: Needleman-Wunsch

- Best global alignment
  - Have maximal alignment score with all bases in two sequences

  - Assume we have two sequences: **P** and **Q**
    - **P** = TCATGGC
    - **Q** = TCATC

    - Score functions

$$\emptyset(a,b) = \begin{cases} 1\ if\ a = b \\ -1\ if\ a \neq b \\ -1\ if\ a = gap \\ -1\ if\ b = gap \end{cases}$$

Needelman & Wunsch, J Mol Biol, 1970

Needleman Wunsch alignment:

- 1. Tabular arrangement
  - $C(0,j) = \sum_{1 \le k \le \|P\|} \emptyset(-, P(k))$
  - $C(i,0) = \sum_{1 \le k \le \|Q\|} \emptyset(Q(k), -)$

$$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

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

Global alignment: Needleman-Wunsch

- 2. Recursive calculation $C(i,j) = max \begin{cases} C(i-1,j-1) + \emptyset(Q(i),P(j)) \\ C(i-1,j) + \emptyset(Q(i),-) \\ C(i,j-1) + \emptyset(-,P(j)) \end{cases}$

$$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

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

Global alignment: Needleman-Wunsch

- 2. Recursive calculation $C(i,j) = max \begin{cases} C(i-1,j-1) + \emptyset(Q(i),P(j)) \\ C(i-1,j) + \emptyset(Q(i),-) \\ C(i,j-1) + \emptyset(-,P(j)) \end{cases}$

$$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

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

1

Global alignment: Needleman-Wunsch

- 2. Recursive calculation $C(i,j) = max \begin{cases} C(i-1,j-1) + \emptyset(Q(i),P(j)) \\ C(i-1,j) + \emptyset(Q(i),-) \\ C(i,j-1) + \emptyset(-,P(j)) \end{cases}$

$$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

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

1

-1

Global alignment: Needleman-Wunsch

- 2. Recursive calculation $C(i,j) = max \begin{cases} C(i-1,j-1) + \emptyset(Q(i),P(j)) \\ C(i-1,j) + \emptyset(Q(i),-) \\ C(i,j-1) + \emptyset(-,P(j)) \end{cases}$

$$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

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

1 -1 -1

Global alignment: Needleman-Wunsch

- 2. Recursive calculation $C(i,j) = max \begin{cases} C(i-1,j-1) + \emptyset(Q(i),P(j)) \\ C(i-1,j) + \emptyset(Q(i),-) \\ C(i,j-1) + \emptyset(-,P(j)) \end{cases}$

$$\emptyset(a,b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; b = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

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

Global alignment: Needleman-Wunsch

- 3. Traceback
  - Check which operation obtained the current alignment score.

Q

P

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

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

Local alignment: Smith-Waterman

- Best local alignment
  - Have maximal alignment score with a subset of bases in two sequences
  - Assume we have two sequences: **P** and **Q**
    - **P** = TCATGGC
    - **Q** = TCATC
    - Score functions

$$\emptyset(a,b) = \begin{cases} 1\ if\ a = b \\ -1\ if\ a \neq b \\ -1\ if\ a = gap \\ -1\ if\ b = gap \end{cases}$$

Smith & Waterman. J Mol Biol. 1981. 147(1):195–197.

Local alignment: Smith-Waterman

- 1. Tabular arrangement
  - $C(0, j) = 0$
  - $C(i, 0) = 0$

$$\emptyset(a, b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

	-	T	C	A	T	G	G	C
-	0	0	0	0	0	0	0	0
T	0							
C	0							
A	0							
T	0							
C	0							

Local alignment: Smith-Waterman

- 2. Tabular arrangement$(i, j) =$
- $max \begin{cases} C(i-1, j-1) + \emptyset(Q(i), P(j)) \\ C(i-1, j) + \emptyset(Q(i), -) \\ C(i, j-1) + \emptyset(-, P(j)) \\ 0 \end{cases}$

$$\emptyset(a, b) = \begin{cases} 1 \; if \; a = b \\ -1 \; if \; a \neq b \\ -1 \; if \; a = gap \\ -1 \; if \; b = gap \end{cases}$$

Q

P

	-	T	C	A	T	G	G	C
-	0	0	0	0	0	0	0	0
T	0	1	0	0	1	0	0	0
C	0	0	2	1	0	0	0	1
A	0	0	1	3	2	1	0	0
T	0	0	0	0	4	3	2	1
C	0	0	0	0	3	3	2	3

Local alignment: Smith-Waterman

- 3. Traceback
  - 1. Find a best score recursively
  - 2. Check which operation is used to obtain the current alignment score.
  - 3. Stop when an alignment is 0

```
T C A T
| | | |
T C A T
```

Q

P

	-	T	C	A	T	G	G	C
-	0	0	0	0	0	0	0	0
T	0	1	0	0	1	0	0	0
C	0	0	2	1	0	0	0	1
A	0	0	1	3	2	1	0	0
T	0	0	0	0	4	3	2	1
C	0	0	0	0	3	3	2	3

BLAST

- BLAST is used for sequence similarity search, and much faster than Smith-Waterman method
- Compare a query sequence against a database of sequences.

- BLAST is a collection of algorithms
  - BLASTN: nucleotide sequence against nucleotide database
  - BLASTP: protein sequence against protein database
  - BLASTX: translated nucleotide sequence against protein database
  - TBLASTX: six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database
  - TBLASTN: protein sequence against translated nucleotide databases

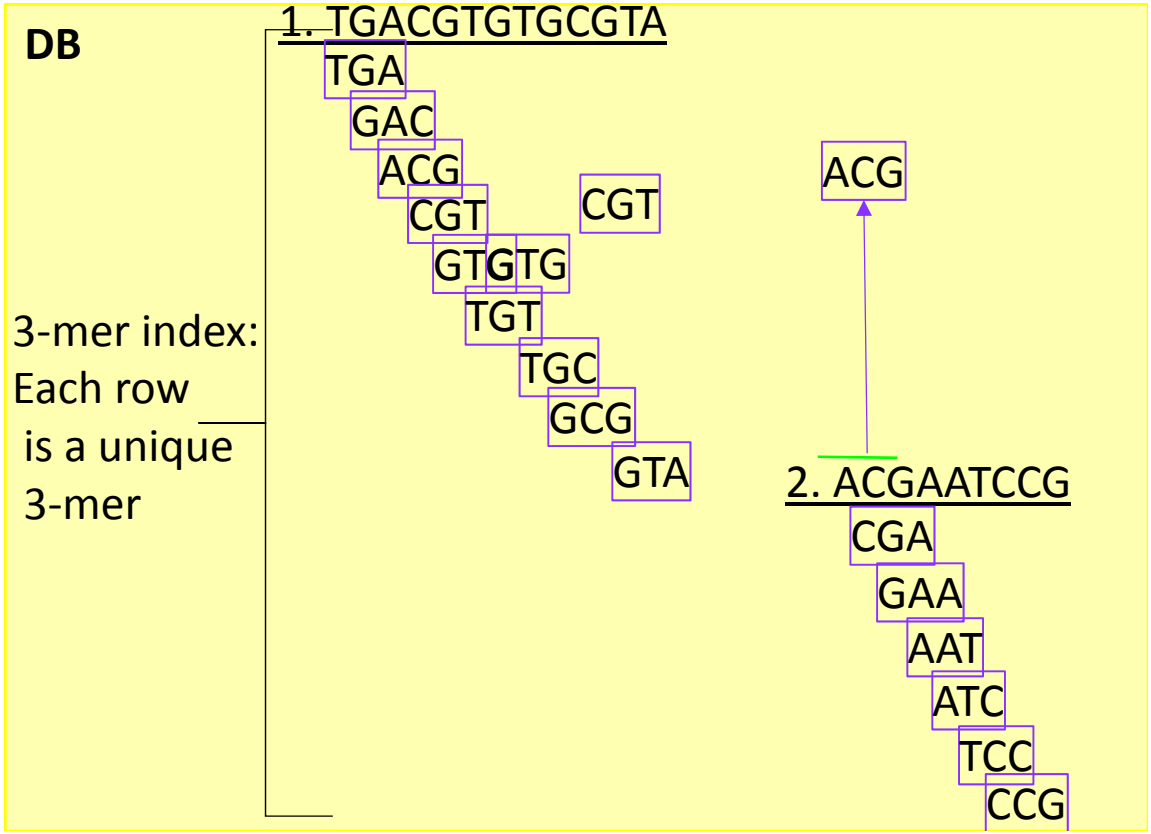
Altschul et al, J Mol Biol. 1990

BLAST

Assume all 3-mer are high-scoring words

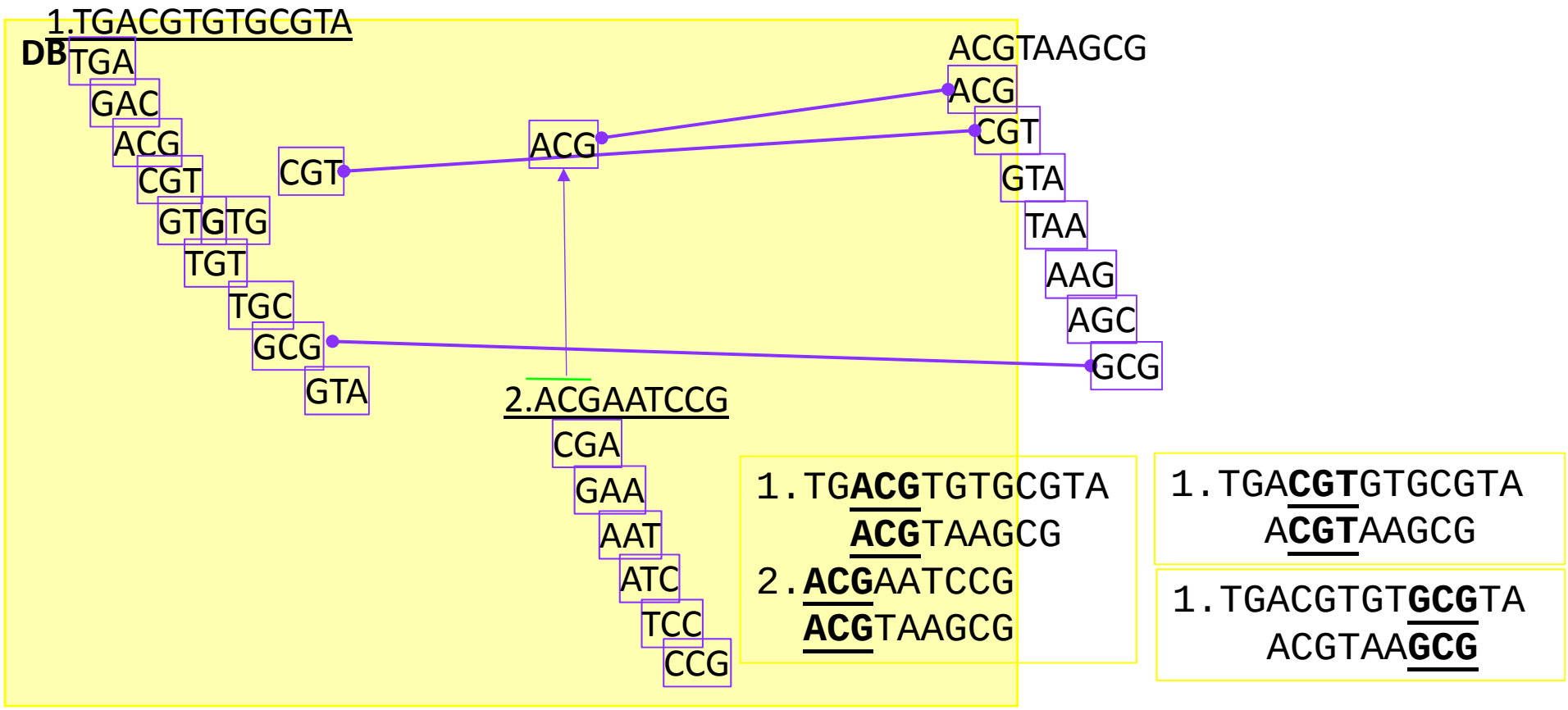
- Process: The seed-index-map-extend-merge strategy
  - Seed and index:
    - Construct common words (k-mers) for sequences in a database
    - Assume the database has two sequences and k=3
      - 1. TGACGTGTGCGTA
      - 2. ACGAATCCG

- Assume all 3-mers are high-score words
  - BLAST needs a threshold to determine high-score words.



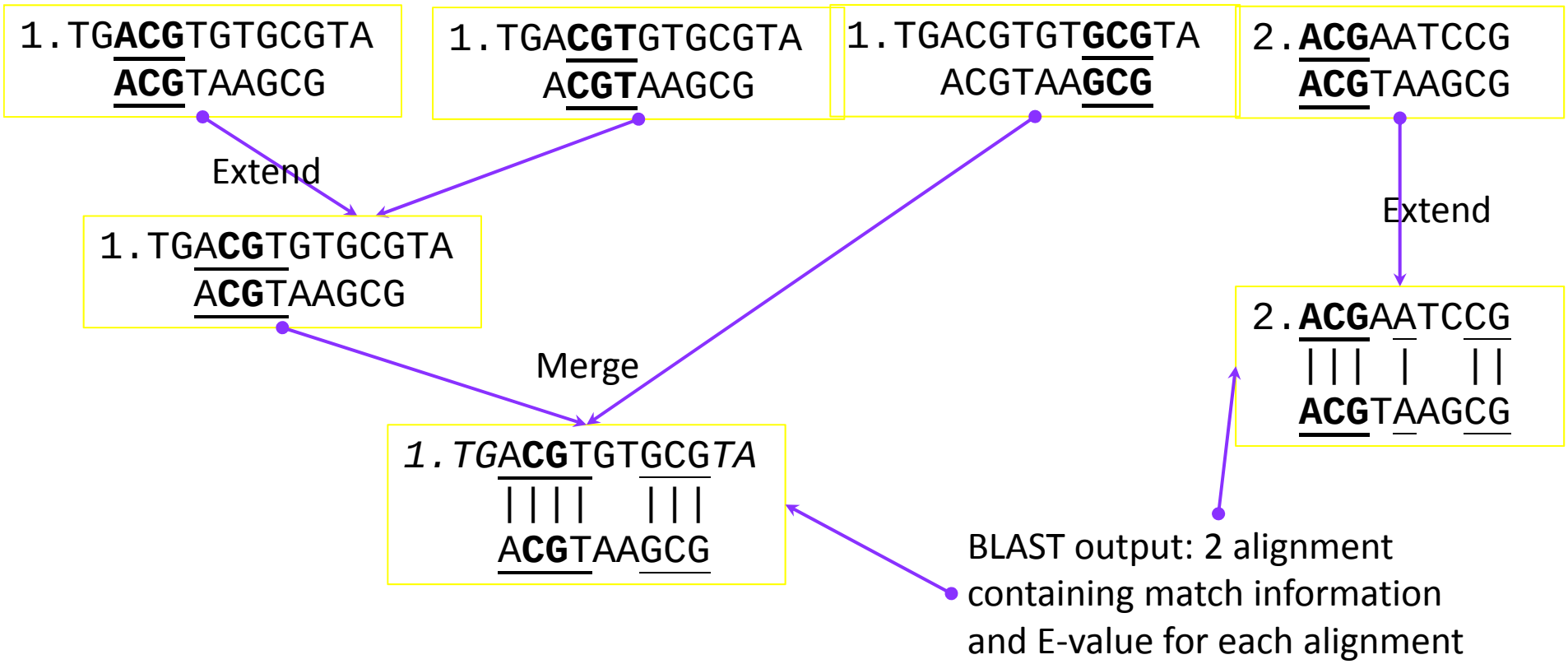
BLAST

- Process: The seed-index-map-extend-merge strategy
  - Seed-map with high-score words
    - Obtain words from a query sequence



BLAST

- Process: The seed-index-map-extend-merge strategy
  - Extend-merge
    - Extend: high-scoring segment



Statistical significance of alignment

- How to assess the significance of a high-scoring hit to the database?
  - E is the number of alignments expected by chance during a database search
  - E is a function of the size of the search space (mn), the normalized score (λS), and a constant (K).
- Karlin-Altschul equation: $E = Kmne^{-\lambda S}$
  - Score (S) from scoring matrix
  - K and λ are two constants
  - m: the length of a query sequence
  - n: the sum of bases of all sequences in database
  - The lower E value indicates more significant alignment.
  - E = − ln(1 − P)

Statistical significance of alignment

- How to assess the P-value of finding a high-scoring pair (HSP)?
  - The number of random HSPs with score >= *S* is described by a Poisson distribution
  - the probability of finding exactly *k* HSPs with score >=*S* is given by $e^{-E} * E^k/k!$
  - Specifically the chance of finding zero HSPs with score >=*S* is e^{-E}, so the probability of finding at least one such HSP is $P=1-e^{-E}$
  - Conversely, $E = -\ln(1 - P)$
  - When E and P are very small, they are almost identical

Bowtie/BWA

- Ultrafast, memory-efficient alignment programs for aligning short DNA sequence reads to large genome
- Burrows–Wheeler transform (BWT)
  - Invented by Burrows and Wheeler, 1994
  - Is a block-sorting compression algorithm for many repeated characters
  - BWA and Bowtie are the most famous implementations of BWT in sequence alignment

Burrows–Wheeler transform (BWT)

- BWT is an invertible transformation of a string *S* of length *n* into another string *S'* of length *n*
- BWT can organize the genome string into a sequence of suffixes of the original genome string
- Given a string *S*, BWT
  - Add $ and assume $<any alphabet
  - Obtains a suffix array of all cyclic rotations
  - Transform into a new string S'

BWT procedures

- Given a string S = “TCATC”, BWT
  - Adds $ and assume $<any alphabet
  - Obtains a suffix array of all cyclic rotations

Transformation				
1. Input	**2. Cyclic rotations**	**3. Sorting**	**4. Last column**	**5. BWT output**
TCATC$				

BWT procedures

- Cyclic rotation:
  - Obtains a suffix array of all cyclic rotations
  - Keeps an index of the rotated strings in the array
  - Creates Circular Permutation Table (CPT)

Transformation				
1. Input	**2. Cyclic rotations**	**3. Sorting**	**4. Last column**	**5. BWT output**
TCATC$	0 TCATC$ 1 CATC$T 2 ATC$TC 3 TC$TCA 4 C$TCAT 5 $TCATC			

BWT procedures

- Sorting:
  - Sorts the Circular Permutation Table (CPT) alphabetically
  - Keep the index with the strings

Transformation				
1. Input	**2. Cyclic rotations**	**3. Sorting**	**4. Last column**	**5. BWT output**
TCATC$	0 TCATC$ 1 CATC$T 2 ATC$TC 3 TC$TCA 4 C$TCAT 5 $TCATC	5 $TCATC 2 ATC$TC 4 C$TCAT 1 CATC$T 3 TC$TCA 0 TCATC$		

BWT procedures

- Taking the last column from the sorted array

Transformation				
1. Input	**2. Cyclic rotations**	**3. Sorting**	**4. Last column**	**5. BWT output**
TCATC$	0 TCATC$ 1 CATC$T 2 ATC$TC 3 TC$TCA 4 C$TCAT 5 $TCATC	5 $TCATC 2 ATC$TC 4 C$TCAT 1 CATC$T 3 TC$TCA 0 TCATC$	5 $TCAT**C** 2 ATC$T**C** 4 C$TCA**T** 1 CATC$**T** 3 TC$TC**A** 0 TCATC**$**	

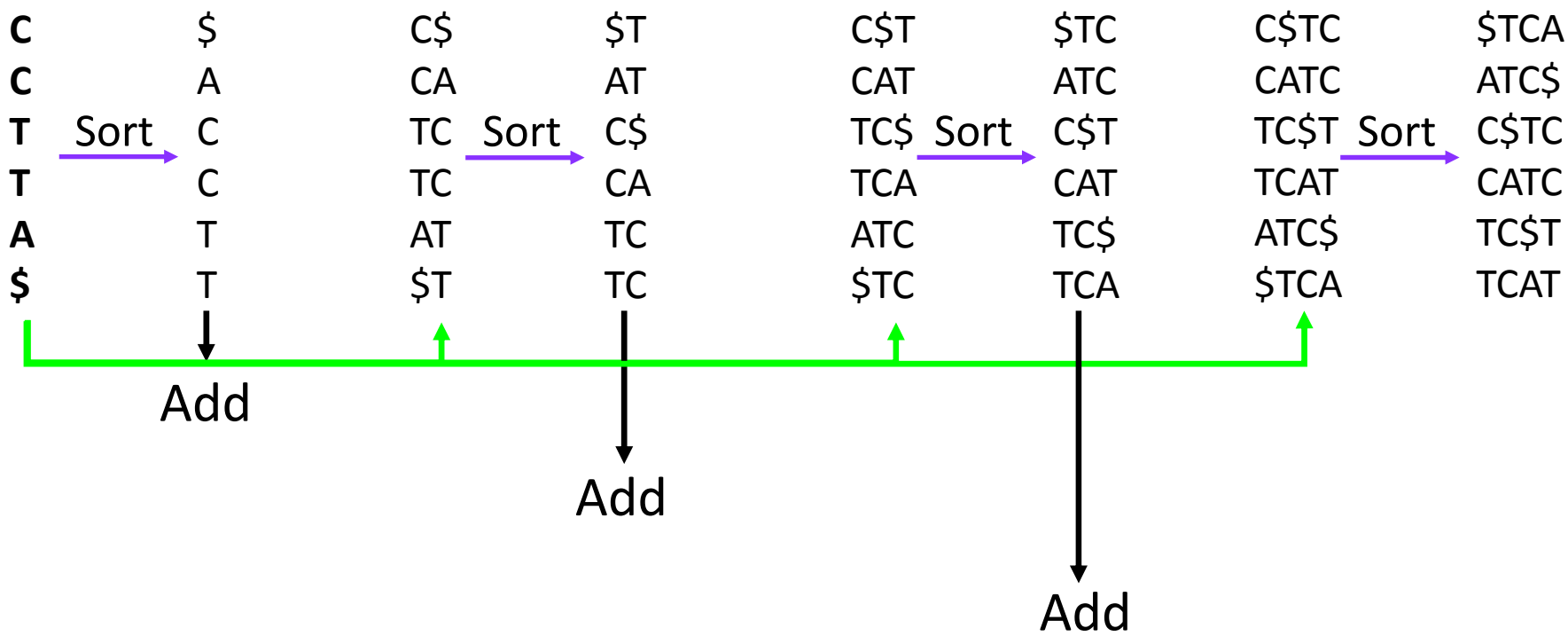
BWT procedures

- BWT output:
  - The last column represents the transformed string

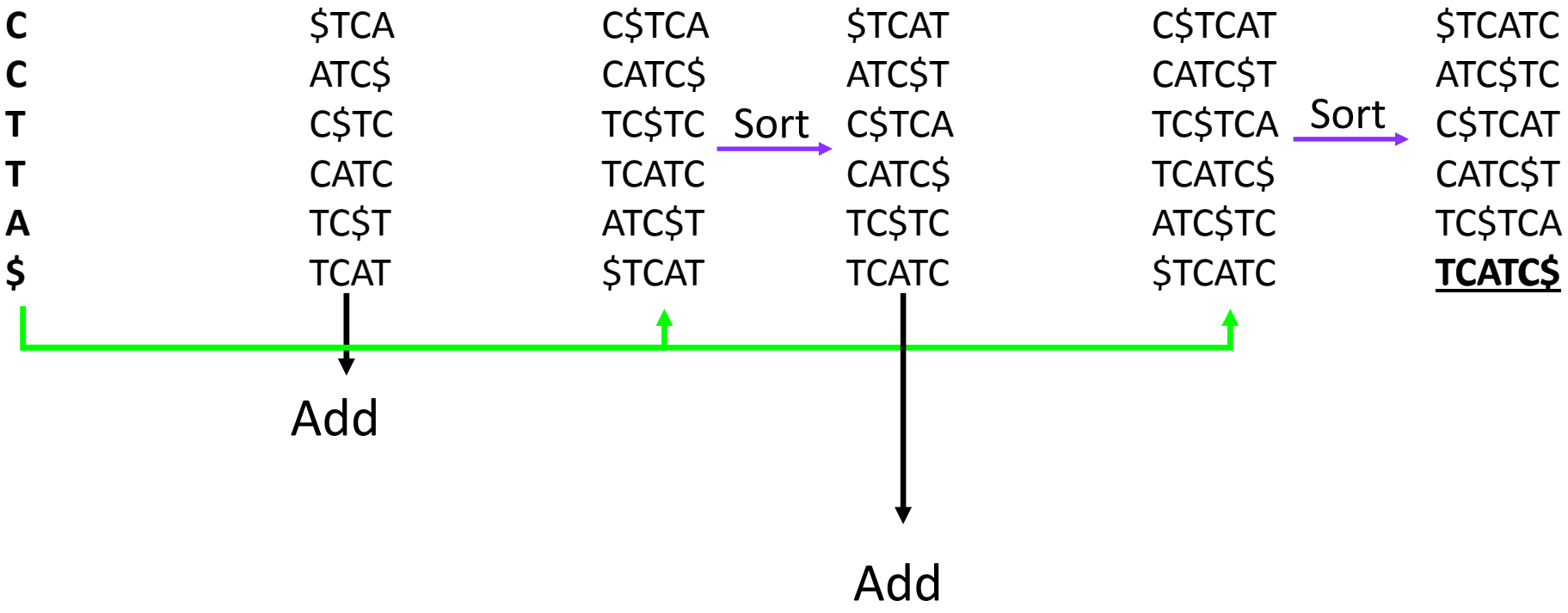
Transformation				
1. Input	**2. Cyclic rotations**	**3. Sorting**	**4. Last column**	**5. BWT output**
TCATC$	0 TCATC$ 1 CATC$T 2 ATC$TC 3 TC$TCA 4 C$TCAT 5 $TCATC	5 $TCATC 2 ATC$TC 4 C$TCAT 1 CATC$T 3 TC$TCA 0 TCATC$	5 $TCAT**C** 2 ATC$T**C** 4 C$TCA**T** 1 CATC$**T** 3 TC$TC**A** 0 TCATC**$**	CCTTA$

BWT reverse transformation

- Transform back to the original string
  - With a suffix array, it is easy to recover the original string S
  - Input: CCTTA$ for TCATC$

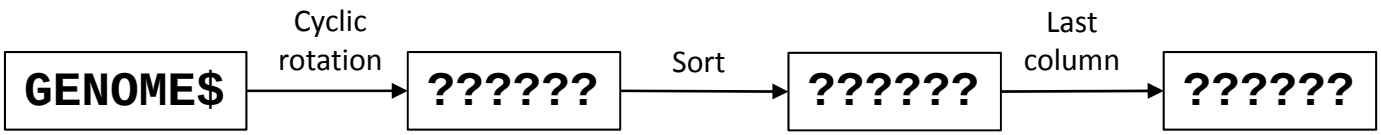


BWT reverse transformation



What's the BWT for GENOME?

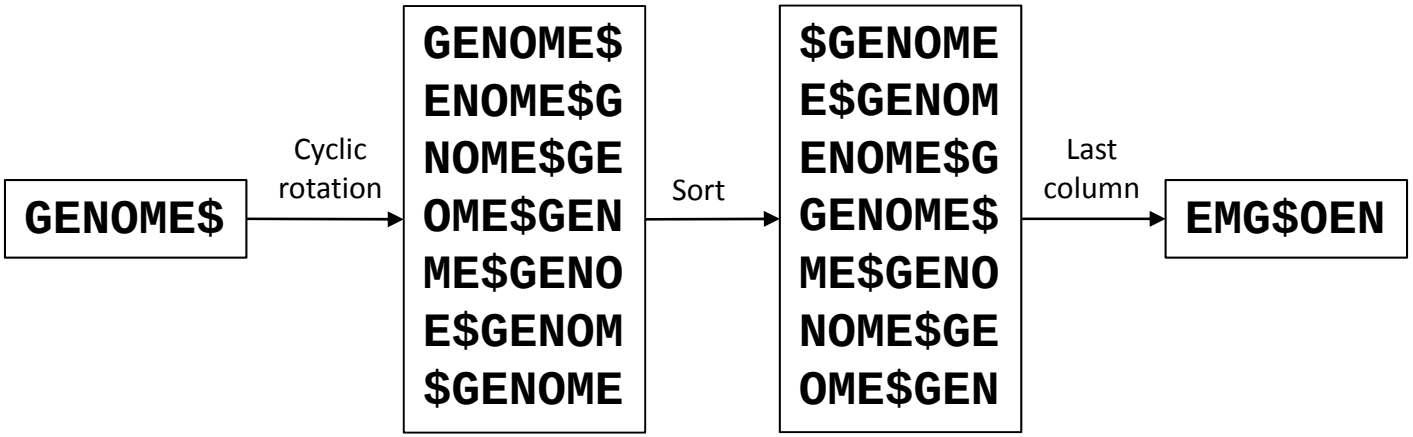
BWT (GENOME$) = ?



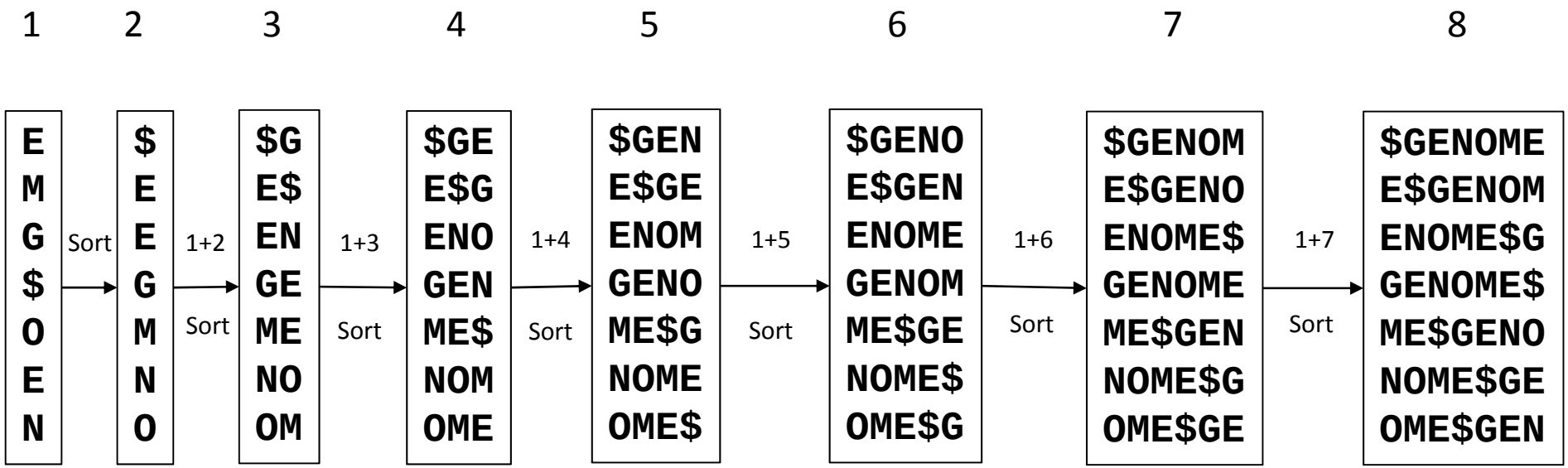
After class exercise: reproduce the BWT transformation above

What's the BWT for GENOME?

BWT (GENOME$) = EMG$OEN



BWT reverse transformation



After class exercise: reproduce the reverse transformation above

BWT search

- Match process:
  - Given a query string, we want to find where is the matched sub-string in original string: TCATC$
  - We do this search through the use of $\mathcal{LF}$ functions on the BWT of the original string
  - $\mathcal{LF}$ (last to first) functions:
    - Purpose: find the same base in the first column corresponding to a specific base in the last column of BWT matrix

LF property

- $\mathcal{LF}$ is a function mapping from the last column of the BWT matrix to the first column
- The ith occurrence of the character X in the last column of BWT matrix corresponds to the ith occurrence of X in the first column.
- Examples:
  - The 1st occurrence of C in the last column is the same C as the 1st occurrence of C in the first column
  - The 2nd occurrence of T in the last column is the same T as the 2nd occurrence of T in the first column

$TCATC
ATC$TC
C$TCAT
CATC$T
TC$TCA
TCATC$

LF calculation

- $\mathcal{LF}$ (pos, na) = $\mathcal{C}$(na) + pre_na(pos, na)
  - pos: position in the output CCTTA$
  - na: a base
  - $\mathcal{C}$(na): the number of bases smaller than na in BWT first column
  - pre_na(pos, na): the number of base na before the position of pos (not included).
- For TCATC (see right side), the $\mathcal{C}$() for $, A, C, T are 0, 1, 2, 4, respectively. This is pre-computed.
- For example, $\mathcal{LF}$ (3, 'T')
  - $\mathcal{C}$('T') = 4, pre_na(3, 'T') = 1
  - Then: $\mathcal{LF}$(3, 'T') = 4+1 = 5
  - The two underlined T in bold (one in the last column and one in the first column) as the same T in the original string.

```
0 $TCATC
1 ATC$TC
2 C$TCAT
3 CATC$T
4 TC$TCA
5 TCATC$
```

T in the last column pos =3 is the same T in first column pos =5

Now, you can try do the same exercise on the C in the last column pos =1

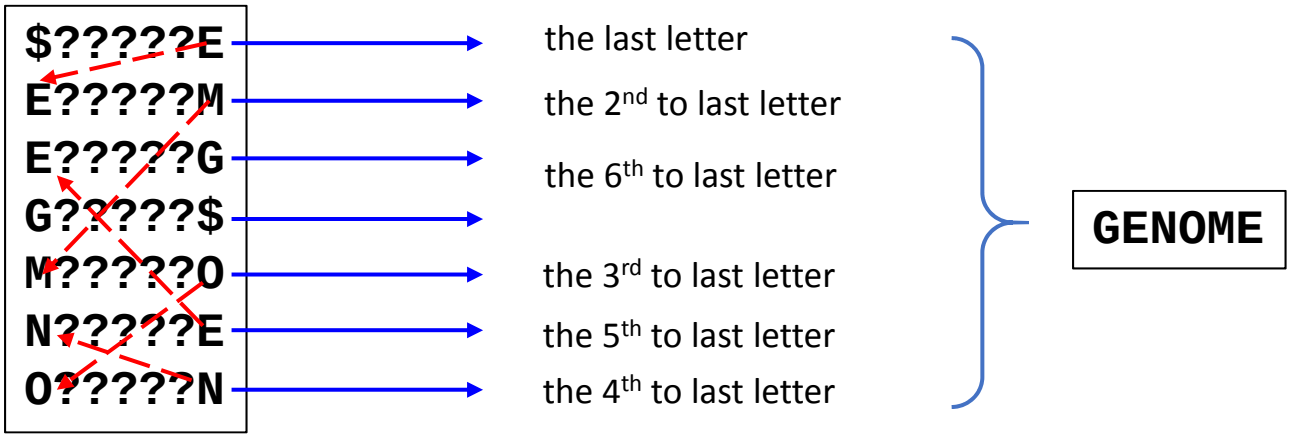
How LF recovers original string

- Suppose we only know first/last column of BWT, what is the original string?

```
$?????E
E?????M
E?????G
G?????$
M?????O
N?????E
O?????N
```

How LF recovers original string

- Suppose we only know first/last column of BWT, what is the original string?



BWT search

- Purpose: search a specific pattern (query) in the BWT matrix, and recover the index in original database sequence
  - Example: search “AT” within the database sequence “TCATC” (the index of “AT” is 2)

0	1	2	3	4	5
T	C	A	T	C	$

- Next we define the lower and upper bound of search recursively (from last to first base of query)
  - L(W): lowest index in BWT matrix where W is prefix
  - U(W): highest index in BWT matrix where W is prefix
  - The L() and U() for A, C, G, T can be pre-calculated from the BWT matrix
  - For a new prefix p in front of W, we will have:
    - L(pW) = $\mathcal{LF}$ (L(W), p)
    - U(pW) = $\mathcal{LF}$ (U(W)+1, p)-1

BWT search

- Match process:
  - Input BWT: CCTTA$
  - Query: AT

C	C	T	T	A	$
0	1	2	3	4	5

- The match process is:
  - 1. for ‘T’ in “AT”
    - L(T) = 4
    - U(T) = 5
  - 2. for ‘A’ in “AT”
    - L(AT) = $\mathcal{LF}$ (L(T),‘A’)=$\mathcal{LF}$ (4, ‘A’)=1+0=1
    - U(AT) = $\mathcal{LF}$ (U(T)+1,‘A’)-1= $\mathcal{LF}$ (6,‘A’)-1=1+1-1=1
  - The matched row is: 1
    - The index in original string is 2

0	5	$TCATC
1	2	ATC$TC
2	4	C$TCAT
3	1	CATC$T
4	3	TC$TCA
5	0	TCATC$

0	1	2	3	4	5
T	C	A	T	C	$

BWT search

- Match process:
  - Input BWT: CCTTA$
  - Query: TC

C	C	T	T	A	$
0	1	2	3	4	5

- The match process is:
  - 1. for ‘C’ in “TC”
    - L(C) = 2
    - U(C) = 3
  - 2. for ‘T’ in “TC”
    - L(TC) = $\mathcal{LF}$ (L(C), ‘T’) =$\mathcal{LF}$ (2, ‘T’)=4+0=4
    - U(TC) = $\mathcal{LF}$ (U(C)+1,‘T’)-1 =$\mathcal{LF}$ (4,‘T’)-1=4+2-1=5
  - The matched row is: 4 and 5.
    - The index in original string is 0 and 3.

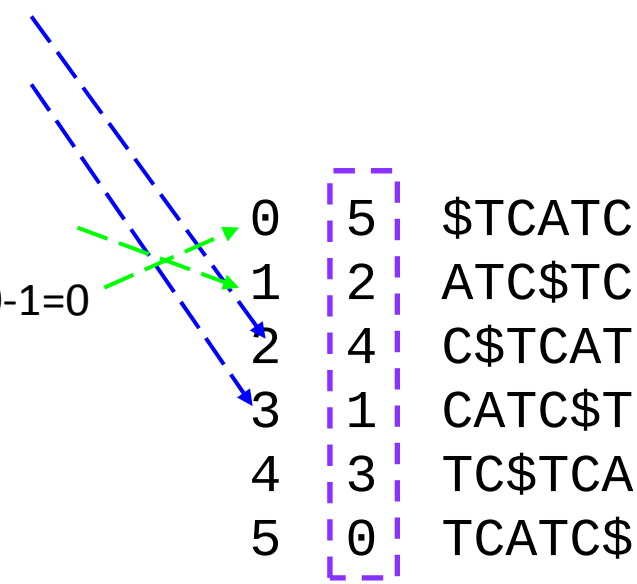
0	5	$TCATC
1	2	ATC$TC
2	4	C$TCAT
3	1	CATC$T
4	3	TC$TCA
5	0	TCATC$

0	1	2	3	4	5
T	C	A	T	C	$

BWT search

- Match process:
  - Input BWT: CCTTA$
  - Query: AC
- The match process is:
  - 1. for ‘C’ in “AC”
    - L(C) = 2
    - U(C) = 3
  - 2. for ‘A’ in “AC”
    - L(AC) = $\mathcal{LF}$ (L(C), ‘A’)= $\mathcal{LF}$ (2, ‘A’)=1+0=1
    - U(AC) = $\mathcal{LF}$ (U(C)+1,‘A’)-1=$\mathcal{LF}$ (4,‘A’)-1 = 1+0-1=0
  - The matched row is None.
    - “AC” is not in “TCATC”

C	C	T	T	A	$
0	1	2	3	4	5

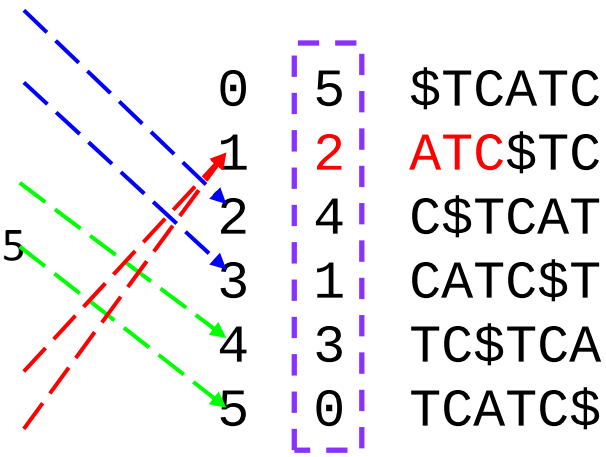


BWT search

- Match process:
  - Input reference: CCTTA$
  - Query: ATC

C	C	T	T	A	$
0	1	2	3	4	5

- The match process is:
  - 1. for ‘C’ in “ATC”
    - L(C) = 2
    - U(C) = 3
  - 2. for ‘T’ in “ATC”
    - L(TC) = $\mathcal{LF}$ (L(C), ‘T’)=$\mathcal{LF}$ (2,‘T’)=4+0=4
    - U(TC) = $\mathcal{LF}$ (U(C)+1,‘T’)-1=$\mathcal{LF}$ (4,‘T’)-1 = 4+2-1=5
  - 3. for “A” in “ATC”
    - L(ATC) = $\mathcal{LF}$ (4, ‘A’) = 1 + 0 = 1
    - U(ATC) = $\mathcal{LF}$ (6, ‘A’)-1 =1+1-1 = 1
  - The matched row is 1
    - The original index is 2



0	1	2	3	4	5
T	C	A	T	C	$

Minimap2

- Fast and accurate aligner for whole genome sequencing data against a reference genome
  - Can work with both short reads and noisy long reads
  - A typical seed-chain-alignment strategy
    - 1. Collect minimizers of reference sequence
    - 2. Index in a hash table
    - 3. Get minimizers from query sequences
    - 4. Find exact match as anchors
    - 5. Find collinear anchors
    - 6. Extend or close gaps by dynamic programming

Li, Bioinformatics, 2018

Traditional k-mers methods vs minimap2

- Traditional k-mer based sequence similarity
  - BLAST:
    - Get k-mer, generate hash value and store in a hash table
    - Find k-mer match between reference sequence and query sequence
  - DALIGNER:
    - Generate k-mer for each of two sets of reads
    - Sort k-mers and merge them for potential match
  - MHAP:
    - *m* k-mer hash functions
    - For each hash functions, find minimum hash value for all k-mers in a sequence
    - Two sequences are similar to each other if they have many overlaps of minimum hash value
- Minimap uses hash functions, but only focuses on “minimizers”, to reduce storage and improve speed substantially

The concept of “minimizer”

- Chooses a representative k-mer from a group of adjacent k-mers, so different strings Ti and Tj choose the same representative if they share a long enough subsequence.
- Only a small fraction of k-mers, called ‘minimizers’, needs to be stored.

Position	1	2	3	4	5	6	7
Sequence	2	3	1	0	3	4	3
k-mers	2	3	1				
with		3	1	0			
minimizer			1	0	3		
in				**0**	**3**	**4**	
bold					3	4	3
	(a)						

Position	1	2	3	4	5	6	7	8	9	10	11	12
Sequence	4	2	6	4	7	2	8	1	4	7	5	1
k-mers	4	2	6	4	7	2	8					
with		**2**	**6**	**4**	**7**	**2**	**8**	**1**				
minimizer			6	4	7	2	8	1	4			
in				4	7	2	8	1	4	7		
bold					7	2	8	1	4	7	5	
	(b)					2	8	1	4	7	5	1

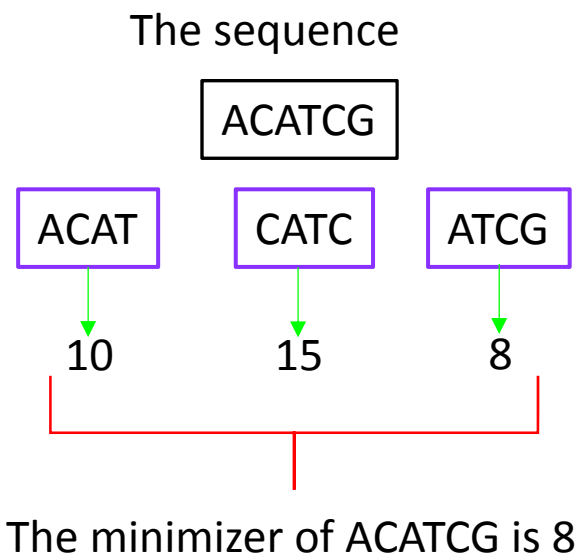
Roberts et al, Bioinformatics, 2004

Minimap2

- 1. Collect minimizers of reference sequence
  - Minimizer:
    - A smallest *k*-mer of *w* consecutive *k*-mers in a sequence with *w+k* bases
    - Minimap2: *k*=15 and *w*=5
  - Example:
    - Assume *w*=3 and *k*=4

three k-mer (4-mer)

hash value



  - Given a sequence with >*w+k* bases
    - A set of minimizers were generated

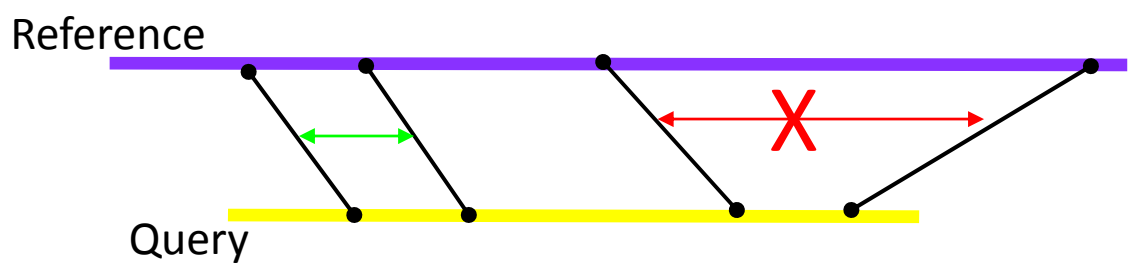
Li, Bioinformatics, 2018

Minimap2

- 2. Index in a hash table
  - Key: a hash value of a minimizer
  - Values: list of locations of minimizer in the reference genome
- 3. Get minimizers from query sequences
  - Used the same hash function to obtain a set of minimizers for query sequence
- 4. Find exact match as anchors
  - Match minimizers between reference and query sequence
  - The matched sub-sequence of reference and sub-sequence of query: anchors

Minimap2

- 5. Find collinear anchors
  - Two anchors are collinear
    - Their distance in a sequence is less than a threshold.
    - Forward and reverse strands are considered individually
    - Cluster close anchors.



- 6. Extend or close gaps between anchors by dynamic programming