Alignment (I)

Bioinformatics Applications (PLPTH813)

Sanzhen Liu

2/25/2019

Review

- FASTA and FASTQ

- Sequence quality (Phred)

Q = $-10 \times \log_{10}(p)$

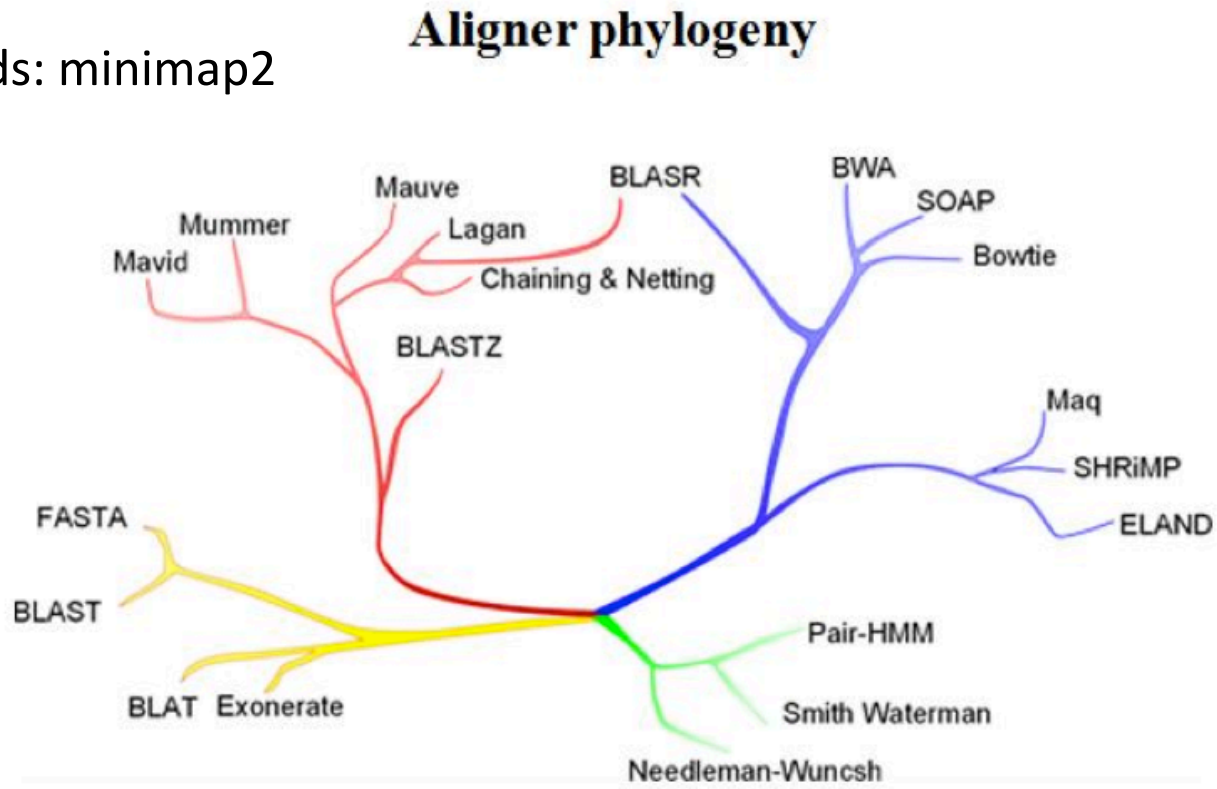
p = $10^{-Q/10}$

- fastQC for quality checking

- Trimmomatic for quality and adaptor trimming

Alignment algorithms

long noisy reads: minimap2



Long sequences	Short reads
Pairwise heuristic	Sensitive aligners

http://evomicsorg.wpengine.netdna-cdn.com/wp-content/uploads/2014/01/alignCompare2.jpg

Outline

- Alignment overview
- Dot plot
- Dynamic alignment

(example: local alignment)

- BLAST

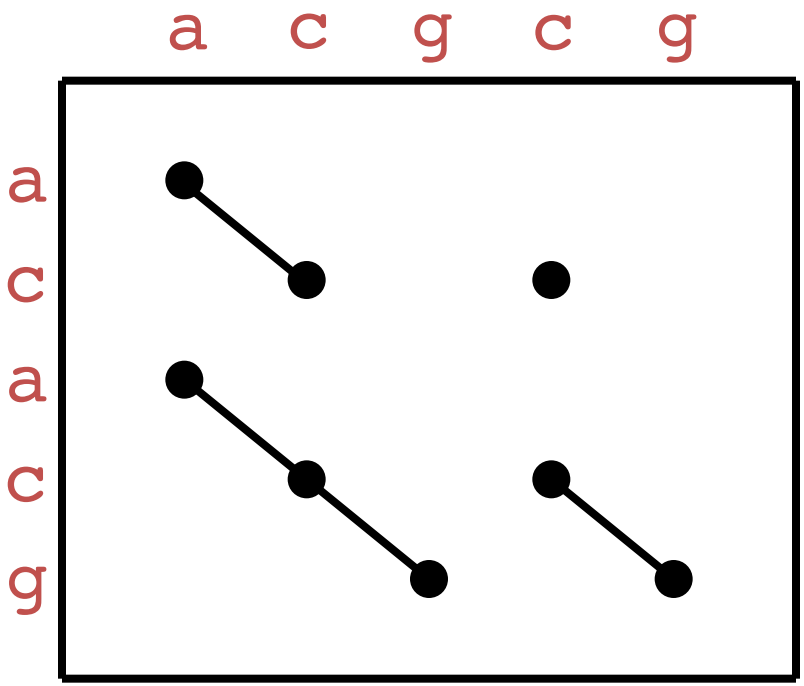
Sequence alignment

Sequence alignment is the approach of comparing the sequences of nucleotides or amino acids to identify regions of similarity.

Applications:

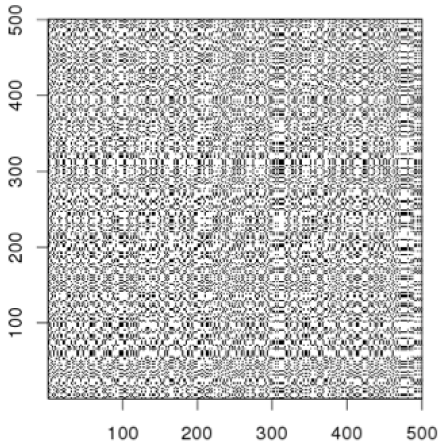
1. Measure relatedness between sequences
2. Identify homologous genes or duplication regions
3. Identify source of a sequence in a database
4. Locate the position of a sequence in the genome
5. etc.

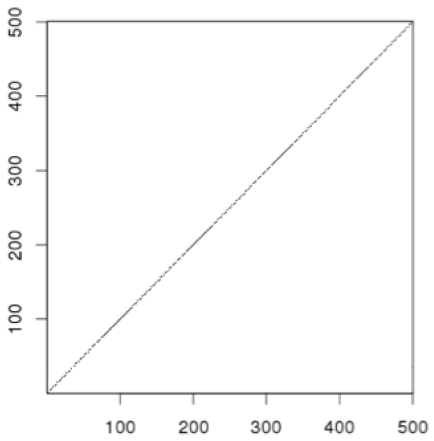
Dot matrices in a single-base resolution



Dot plot comparison using windows

- Dot matrices for long sequences can be noisy due to insignificant matches





e.g., Put a dot/line only if at least 9 out of 10 nucleotides are identical.

window size = 10

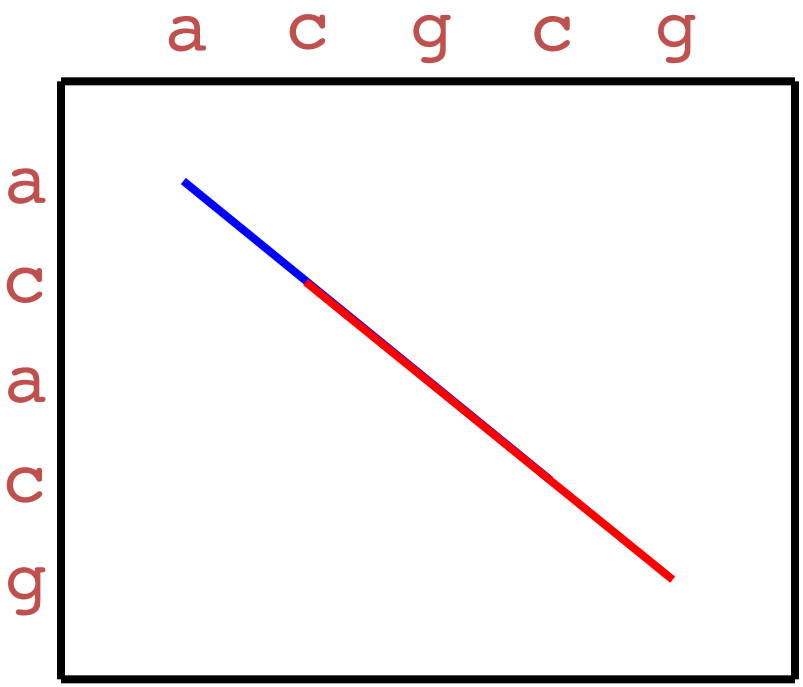
min matches = 9

- Solution: use a window and a threshold
  - compare letter by letter within a window (have to choose window size)
  - require certain fraction of matches within window in order to display it with a dot

Dot plot with a window method

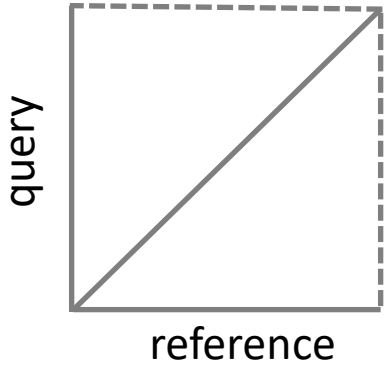
Window size = 4

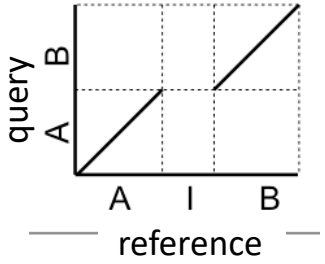
Stringency = 3 (min matches)



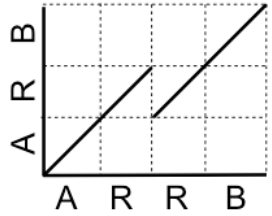
Dot-plots (examples)

query is identical to reference
and contains no repeats

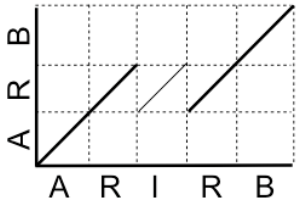




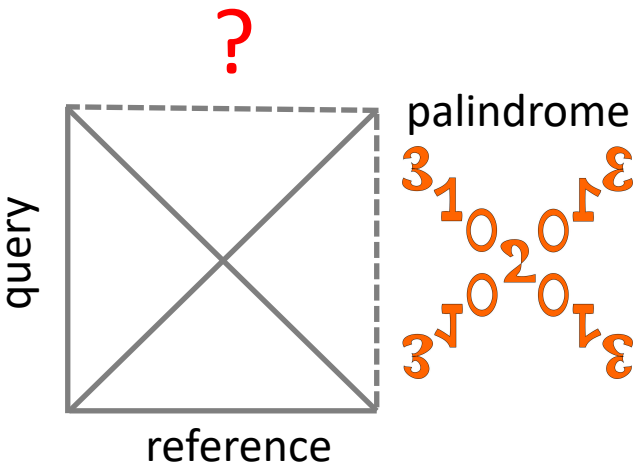
deletion of "I" in query

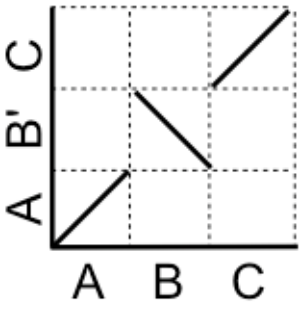


deletion of a "R" in query

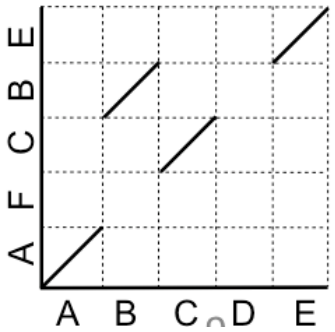


deletion of "IR" in query





inversion



Rearrangement
with disagreement

Outline

- Alignment overview
- Dot plot
- Dynamic alignment

(example: local alignment)

- BLAST

Local and global alignments

- Local alignment: to find similar sequence regions between sequences

```
C  T  G  T  T  G  C  T  G  C
         T  G  C  T  G
```

- Global alignment: to attempt to optimally align the entire length of two sequences.

```
C  T  G  T  T  G  C  T  G  C
-  T  G  -  -  -  C  T  G  -
```

Best (local) alignment

Question: How to determine which alignment is better?

```
Alignment 1: C  T  G  T  T  G  C  T  G  C
                T  G  C  T  G

Alignment 2: C  T  G  T  T  G  C  T  G  C
                T  G  -  -  -  C  T  G
```

Need a scoring scheme:

e.g., match +1; mismatch -1; gap -2

then, a score can be assigned to each alignment

Best (local) alignment

match +1; mismatch -1; gap -2

```
Alignment 1: C  T  G  T  T  G  C  T  G  C
             T  G  C  T  G
             1  2  1  2  3                 score = 3
Alignment 2: C  T  G  T  T  G  C  T  G  C
             T  G  -  -  -  C  T  G
             1  2  0 -2 -4 -3 -2 -1        score = -1
```

match +1; mismatch **-2**; gap **0**

```
Alignment 1: C  T  G  T  T  G  C  T  G  C
             T  G  C  T  G
             1  2  0  1  1                 score = 2
Alignment 2: C  T  G  T  T  G  C  T  G  C
             T  G  -  -  -  C  T  G
             1  2  0  0  0  3  4  5        score = 5
```

A classic algorithm for local alignment – Smith-Waterman

Local alignment

```
C  T  G  T  T  G  C  T  G  C
         T  G  C  T  G
```

List all possible alignments and to find the winner with the highest score?

Smith–Waterman (SW)

Using dynamic programming to find the best local alignment(s) between two sequences with respect to a scoring scheme

SW example

Question: to find the optimal local alignments between s and t.

```
s: CTGTTGCT
t: ATGCTGCA
```

Scoring rule:
match +1; mismatch -1; gap -2 (γ)

1. Initialize top row and leftmost column to zero.

2. Fill in the table using the following formula

$C[i-1, j-1]$	$C[i-1, j]$
$C[i, j-1]$	$C[i, j]$

$$C[i,j] = \max \begin{cases} C[i-1,j-1] + score(s[i], t[j]) \\ C[i-1,j] - \gamma \\ C[i,j-1] - \gamma \\ 0 \end{cases}$$

s

	j	1	2	3	4	5	6	7	8	9
i			C	T	G	T	T	G	C	T
1		0	0	0	0	0	0	0	0	0
2	A	0								
3	T	0								
4	G	0								
5	C	0								
6	T	0								
7	G	0								
8	C	0								
9	A	0								

t

SW example

Scoring rule: $score(s,t)$
match +1; mismatch -1; gap -2 (γ)

1. Initialize top row and leftmost column to zero.

2. Fill in the table using the following formula

$C[i\text{-}1, j\text{-}1]$	$C[i\text{-}1, j]$
$C[i, j\text{-}1]$	$C[i, j]$

$$C[i,j] = \max \begin{cases} C[i-1,j-1] + score(s[i], t[j]) & \text{0 - 1} \\ C[i-1,j] - \gamma & \text{0 - 2} \\ C[i,j-1] - \gamma & \text{0 - 2} \\ 0 & \text{0} \end{cases}$$

s

t

	j	1	2	3	4	5	6	7	8	9
i			C	T	G	T	T	G	C	T
1		0	0	0	0	0	0	0	0	0
2	A	0	0							
3	T	0								
4	G	0								
5	C	0								
6	T	0								
7	G	0								
8	C	0								
9	A	0								

SW example

Scoring rule: $score(s,t)$
match +1; mismatch -1; gap -2 (γ)

1. Initialize top row and leftmost column to zero.

2. Fill in the table using the following formula

$C[i\text{-}1, j\text{-}1]$	$C[i\text{-}1, j]$
$C[i, j\text{-}1]$	$C[i, j]$

$$C[i,j] = \max \begin{cases} C[i-1,j-1] + score(s[i], t[j]) \\ C[i-1,j] - \gamma \\ C[i,j-1] - \gamma \\ 0 \end{cases}$$

		j	1	2	3	4	5	6	7	8	9
							s				
i				C	T	G	T	T	G	C	T
1			0	0	0	0	0	0	0	0	0
2	A		0	0	0						
3	T		0	0	1						
4	G		0								
t	5	C	0								
6	T		0								
7	G		0								
8	C		0								
9	A		0								

http://rna.informatik.uni-freiburg.de/Teaching/index.jsp?toolName=Smith-Waterman

SW example (cont.)

Question: to find the optimal local alignments between s and t.

```
s: CTGTTGCT
t: ATGCTGCA
```

	j	1	2	3	4	5	6	7	8	9
i			C	T	G	T	T	G	C	T
1		0	0	0	0	0	0	0	0	0
2	A	0	0	0	0	0	0	0	0	0
3	T	0	0	1	0	1	1	0	0	1
4	G	0	0	0	2	0	0	2	0	0
5	C	0	1	0	0	1	0	0	3	1
6	T	0	0	2	0	1	2	0	1	4
7	G	0	0	0	3	1	0	3	1	2
8	C	0	1	0	1	2	0	1	4	2
9	A	0	0	0	0	0	1	0	2	3

SW example (cont.)

Question: to find the optimal local alignments between s and t.

```
s: CTGTTGCT
t: ATGCTGCA
```

To obtain the optimum local alignment,

3. Identify the highest scores in the matrix.

4. Then, go backwards to the cell with the highest score of the positions of (i – 1, j), (i, j – 1), and (i – 1, j – 1)

5. This procedure is repeated until a cell with zero value is reached.

	j	1	2	3	4	5	6	7	8	9
i			C	T	G	T	T	G	C	T
1		0	0	0	0	0	0	0	0	0
2	A	0	0	0	0	0	0	0	0	0
3	T	0	0	1	0	1	1	0	0	1
4	G	0	0	0	2	0	0	2	0	0
5	C	0	1	0	0	1	0	0	3	1
6	T	0	0	2	0	1	2	0	1	4
7	G	0	0	0	3	1	0	3	1	2
8	C	0	1	0	1	2	0	1	4	2
9	A	0	0	0	0	0	1	0	2	3

```
s: CTGTTGCT      s: CTGTTGCT
    ||||||           ||||
t: ATGCTGCA      t: ATGCTGCA
```

Global alignment – Needleman-Wunsch

Global alignments attempt to optimally align the entire length of two sequences.

+10 for match, -2 for mismatch, -5 for gap

```
s: CTGTTGCT
t: ATGCTGCA
```

$$C[i,j] = \max \begin{cases} C[i-1,j-1] + s(s[i],t[j]) \\ C[i-1,j] - \gamma \\ C[i,j-1] - \gamma \end{cases}$$

t

s

j	1	2	3	4	5	6	7	8	9
i		C	T	G	T	T	G	C	T
1	0	-5	-10	-15	-20	-25	-30	-35	-40
2 A	-5	-2	-7	-12	-17	-22	-27	-32	-37
3 T	-10	-7	8	3	-2	-7	-12	-17	-12
4 G	-15	-12	3	18	13	8	3	-2	-7
5 C	-20	-5	-2	13	16	11	6	13	8
6 T	-25	-10	5	0	23	26	21	16	23
7 G	-30	-15	0	15	18	21	36	31	26
8 C	-35	-20	-5	10	13	16	31	46	41
9 A	-40	-25	-10	5	8	11	26	41	44

```
s: CTG-TTGCT
     ||   |||
t: ATGC-TGCA
```

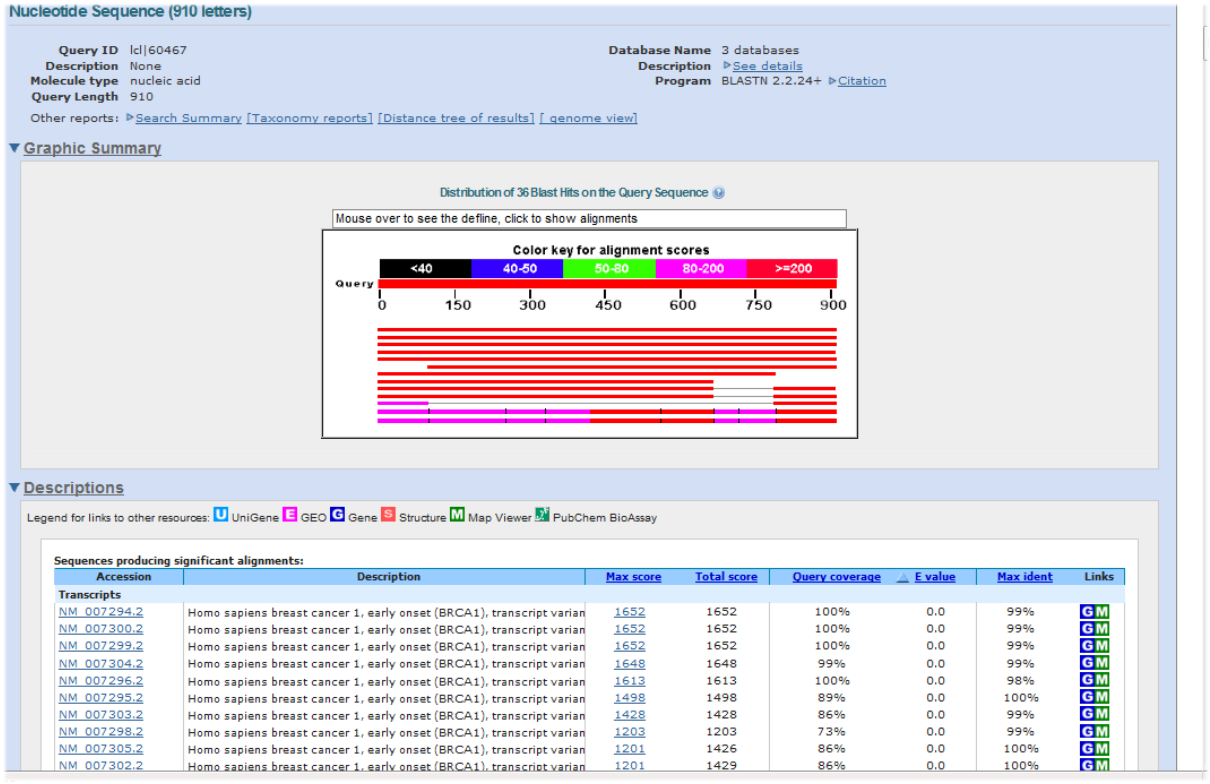
BLAST (Basic Local Alignment Search Tool)

- The classic algorithm, Smith–Waterman algorithm, optimizes the similar measure. It ***ensured*** the *best performance on accuracy* and the most precise results with respect to *its scoring scheme*.

- However, Smith–Waterman algorithm is *time-consuming* and computational burdensome. It is not practical to apply it to align a query sequence to a large database.

- *BLAST* emphasizes on speed to make the algorithm practical on huge genome databases.

Question



Could you recall the procedure of a BLAST job to achieve the BLAST alignment results? And what does NCBI actually provide?

BLAST+

- BLAST was first introduced by NCBI in 1989.
- NCBI introduced BLAST+ in 2009, which is faster and allows more flexibility in output formats and in the search input.
- It provides a variety of BLAST functions for both DNA and protein sequences.

For example:

blastp

blastp	Traditional BLASTP to compare a protein query to a protein database
blastp-short	BLASTP optimized for queries shorter than 30 residues

blastn

blastn	Traditional BLASTN requiring an exact match of 11
blastn-short	BLASTN program optimized for sequences shorter than 50 bases

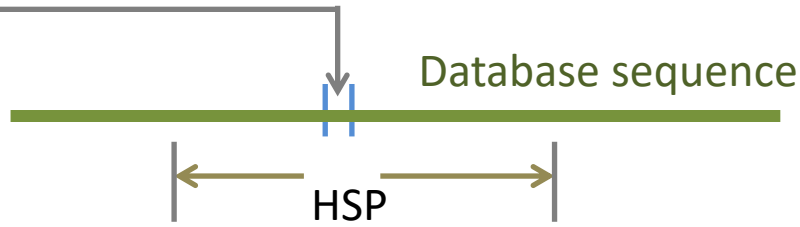
BLAST algorithm

1. Make k-tuple words (seeds) of the query sequence.

```
CTGTTGCTCGTCTCGGGACTGT
CTG
 TGT
  GTT
   …
```

2. List possible matching words for **each k-tuple word** & remove low-scoring words

```
k   mismatch
CTG 0
ATG 1
TTG 1   high-scoring words
GTG 1
CAG 1
…
AAG 2
…
```



3. Compare the high-scoring words to the database sequences to identify exact matches

4. Extend the exact matches to both directions on the database sequences to obtain high-scoring segment pairs (HSPs)

Command line based BLAST

Step 1:

- Computer/server
- Install the “BLAST+” software package
- Make databases of collected sequences

Step 2:

Run BLAST searching with your query sequences on the server

Step 1: Create a database

- **makeblastdb**

A program to create a BLAST database

```
makeblastdb –in MG1655.fasta -out MG1655 -dbtype nucl
```

Database files were generated:

```
---output---
MG1655.nhr
MG1655.nin
MG1655.nsq
...
```

Step 2: BLAST a query to a DNA database

- **blastn**

blastn -query MG1655dnaseq.fa –db MG1655

---output---

```
Query= MG1655_partial
Length=280
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value
gi|556503834|ref|NC_000913.3| Escherichia coli str. K-12 substr...    518   1e-147

> gi|556503834|ref|NC_000913.3| Escherichia coli str. K-12 substr.
MG1655, complete genome
Length=4641652

 Score =   518 bits (280),  Expect = 1e-147
 Identities = 280/280 (100%), Gaps = 0/280 (0%)
 Strand=Plus/Plus

Query  1      TAGAAAATGCCCATGGCAAGAATAATACCGTCCAGAGCGAAATAACCCACGTTGTGCAGG  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10361  TAGAAAATGCCCATGGCAAGAATAATACCGTCCAGAGCGAAATAACCCACGTTGTGCAGG  10420

Query  61     TTAAGCAGAATGGTGGTCATGCCGAAGCCCATCAGGCCCAGCGGTGCCGGATTAGCCAAC  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10421  TTAAGCAGAATGGTGGTCATGCCGAAGCCCATCAGGCCCAGCGGTGCCGGATTAGCCAAC  10480

Query  121    TTAGTGTTGCCCATAATTCCTCAAAAATCATCATCGAATGAATGGTGAAATAATTTCCCT  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10481  TTAGTGTTGCCCATAATTCCTCAAAAATCATCATCGAATGAATGGTGAAATAATTTCCCT  10540

Query  181    GAATAACTGTAGTGTTTTCAGGGCGCGGCATAATAATCAGCCAGTGGGGCAGTGTCTACG  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10541  GAATAACTGTAGTGTTTTCAGGGCGCGGCATAATAATCAGCCAGTGGGGCAGTGTCTACG  10600

Query  241    ATCTTTTGAGGGGAAAATGAAAATTTTCCCCGGTTTCCGG  280
              |||||||||||||||||||||||||||||||||||||||||
Sbjct  10601  ATCTTTTGAGGGGAAAATGAAAATTTTCCCCGGTTTCCGG  10640
```

Select output format

```
blastn -query MG1655dnaseq.fa -db MG1655 -outfmt 6
```

query id	subject id	% identity	alignment length	mismatches	gap opens	q. start	q. end	s. start	s. end	evalue	bit score
MG1655_partial	gi|556503834|ref|NC_000913.3|	100	280	0	0	1	280	10361	10640	1.00E-147	518

E-value

```
Score =   518 bits (280),  Expect = 1e-147
Identities = 280/280 (100%), Gaps = 0/280 (0%)
Strand=Plus/Plus

Query  1      TAGAAAATGCCCATGGCAAGAATAATACCGTCCAGAGCGAAATAACCCACGTTGTGCAGG  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10361  TAGAAAATGCCCATGGCAAGAATAATACCGTCCAGAGCGAAATAACCCACGTTGTGCAGG  10420

…

Query  241    ATCTTTTGAGGGGAAAATGAAAATTTTCCCCGGTTTCCGG  280
              ||||||||||||||||||||||||||||||||||||||||
Sbjct  10601  ATCTTTTGAGGGGAAAATGAAAATTTTCCCCGGTTTCCGG  10640
```

- **E-value** is a parameter that describes the number of hits that one can "expect" to see by chance when searching a database of a particular size. It is used to describe the significance (instead of a p-value) of each sequence alignment hit.

For example, E-value = 1 means that in a database of the similar size 1 match with a similar score would be obtained simply by chance.

- The lower the E-value, the more "significant" the match is.

Score and Bit scores

```
Score =   518 bits (280),  Expect = 1e-147
Identities = 280/280 (100%), Gaps = 0/280 (0%)
Strand=Plus/Plus

Query  1      TAGAAAATGCCCATGGCAAGAATAATACCGTCCAGAGCGAAATAACCCACGTTGTGCAGG  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10361  TAGAAAATGCCCATGGCAAGAATAATACCGTCCAGAGCGAAATAACCCACGTTGTGCAGG  10420

…

Query  241    ATCTTTTGAGGGGAAAATGAAAATTTTCCCCGGTTTCCGG  280
              ||||||||||||||||||||||||||||||||||||||||
Sbjct  10601  ATCTTTTGAGGGGAAAATGAAAATTTTCCCCGGTTTCCGG  10640
```

- In the context of sequence alignments, a score is a numerical value that describes the overall quality of an alignment.
- The **bit-score** is a rescaled alignment score to indicate the alignment quality, which is independent of the size of the search database.
- The higher the score/bit-score, the better alignment is.

Extract sequences or subsequences

- **blastdbcmd**

Extract sequences from the database

```
# Use Gi ID to search*

blastdbcmd –db MG1655 -entry 556503834 -range 150-220


---output---
>gi|556503834|ref|NC_000913.3|:150-220 Escherichia coli str. K-12 substr. MG1655, complete genome
AGCGCACAGACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTAC
CACCA
```

* Database formatting needs to be a little different:

```
makeblastdb –in MG1655.fasta -out MG1655 -dbtype nucl –parse_seqids
```

BLAST tools

blastp: protein blast search

blastx: search protein databases using a translated nucleotide query

tblastn: search translated nucleotide databases using a protein query

tblastx: search translated nucleotide databases using a translated nucleotide query)