

BLAST

Basic Local Alignment Search Tool

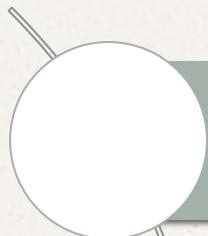
So useful – it is now a verb in the literature



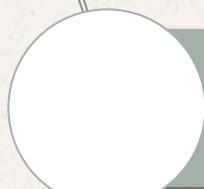
Bob Fitak



Goals for Today:



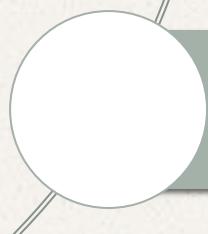
What is BLAST and why is it important?



Principles of the algorithm



Online Examples

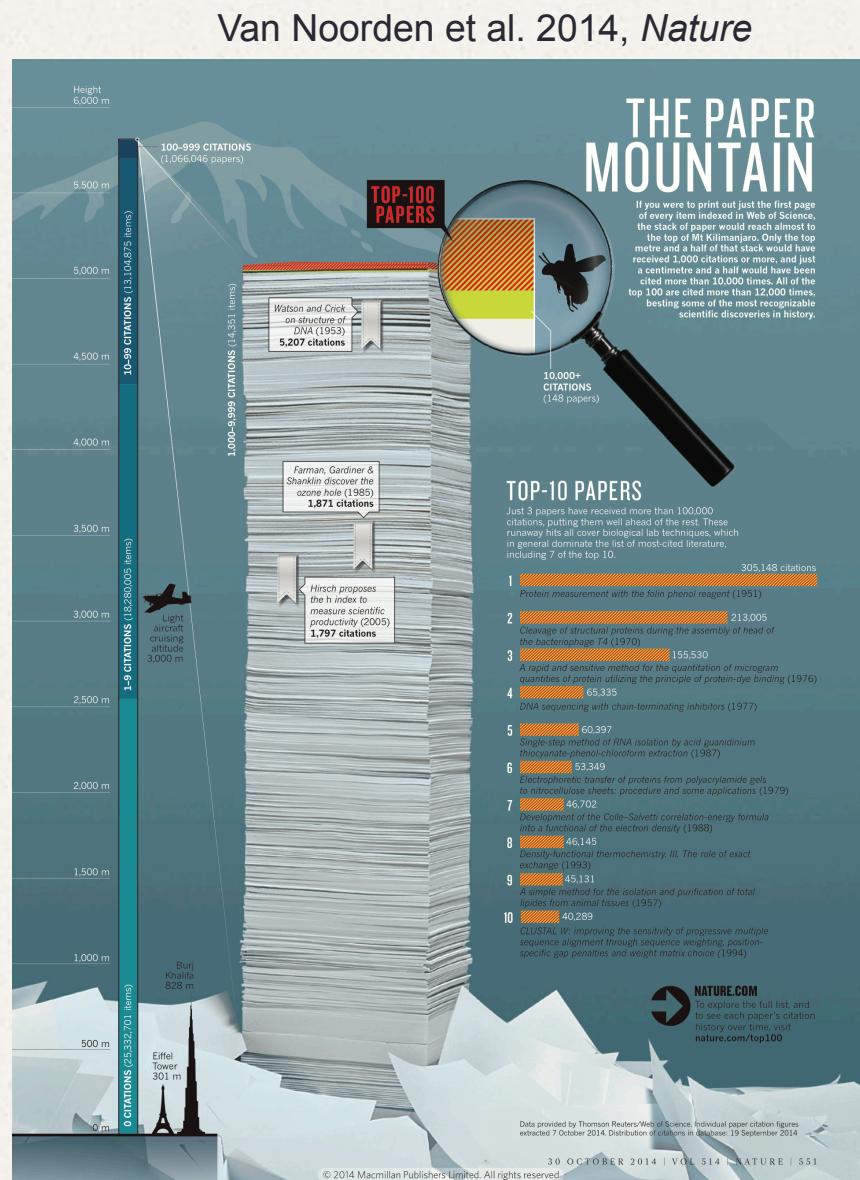


Afternoon: Command line & Geneious



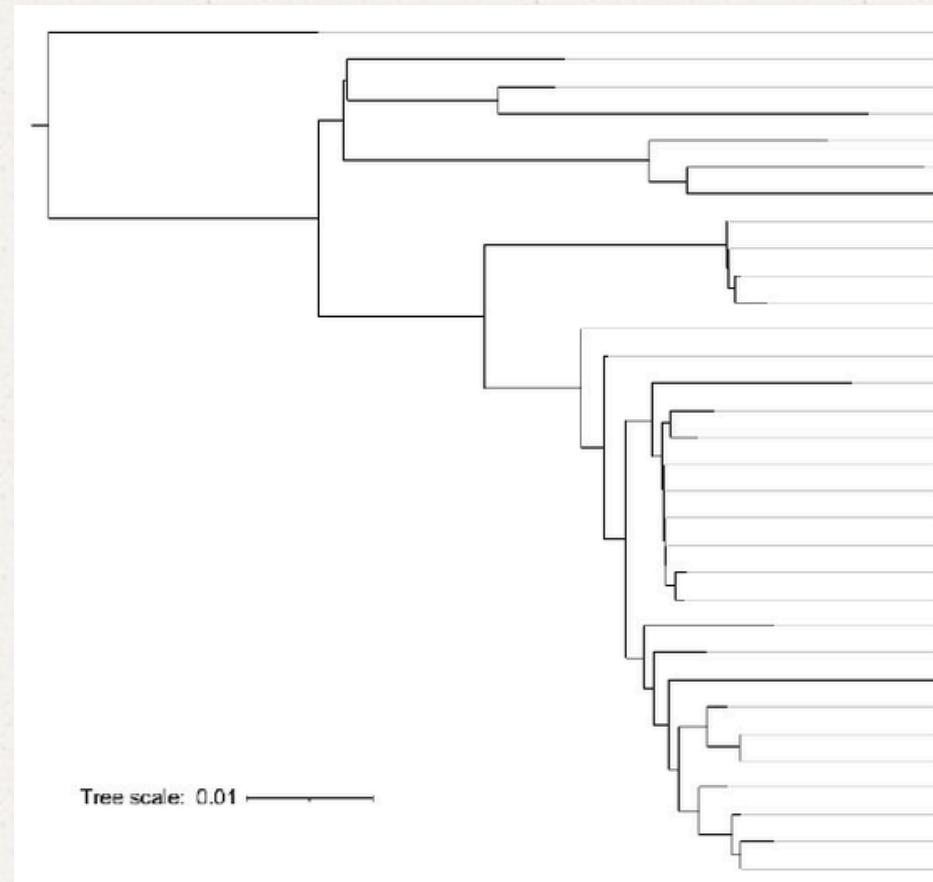
A Lot of Blasting

- Where is BLAST on this list?
 - Altschul et al. 1990
 - #12 – 38,380 citations
 - Altschul et al. 1997
 - #14 – 36,410 citation
 - Combined: 4th!



Goals

- Search a query against a database
 - Identify species
 - Locate domains
 - Assess function
 - Establish phylogeny
 - Mapping



What is BLAST?

- Sequence searching algorithm
- Finds the best local alignments
- Calculates statistical significance
- Similarity suggests homology
- Less sensitive than Smith-Waterman, but FASTER!

Local vs Global Alignment

- Global alignment: entire sequences



- Local alignment: segments of sequences



- Local alignment often the most relevant
 - Depends on biological assumptions



Blast Types

Name	Query	Database
blastn	nucleotide	nucleotide
blastp	protein	protein
blastx	nucleotide	protein
tblastx	nucleotide	nucleotide
tblastn	protein	nucleotide
PSI-blast	protein	protein



Blast Databases: Protein

Name	Host	Description
nr	NCBI	Non-redundant, general
Refseq_protein	NCBI	Annotated and curated protein collection
SwissProt	SIB	Manually curated and reviewed proteins form UniProt
Trembl	EBI	Automatically annotated, non-reviewed proteins
PDB	Rutgers/ UCSD/ UCSC	Proteins with 3D structural information



Blast Databases: Nucleotide

Name	Host	Description
nt	NCBI	Non-redundant, general
Refseq_RNA	NCBI	Annotated and curated RNA sequence collection
Refseq_Genomics	NCBI	Sequenced and curated genomes
EST	NCBI	Expressed sequence tags
UNIVEC	NCBI	Vector contaminant database
WGS	NCBI	Draft, whole genome shotgun sequence assemblies
SRA	NCBI	Raw NGS datasets
Many more databases, e.g. barcoding, viral, tRNA, etc), custom-built databases		



How it works: Making words

Nucleotide

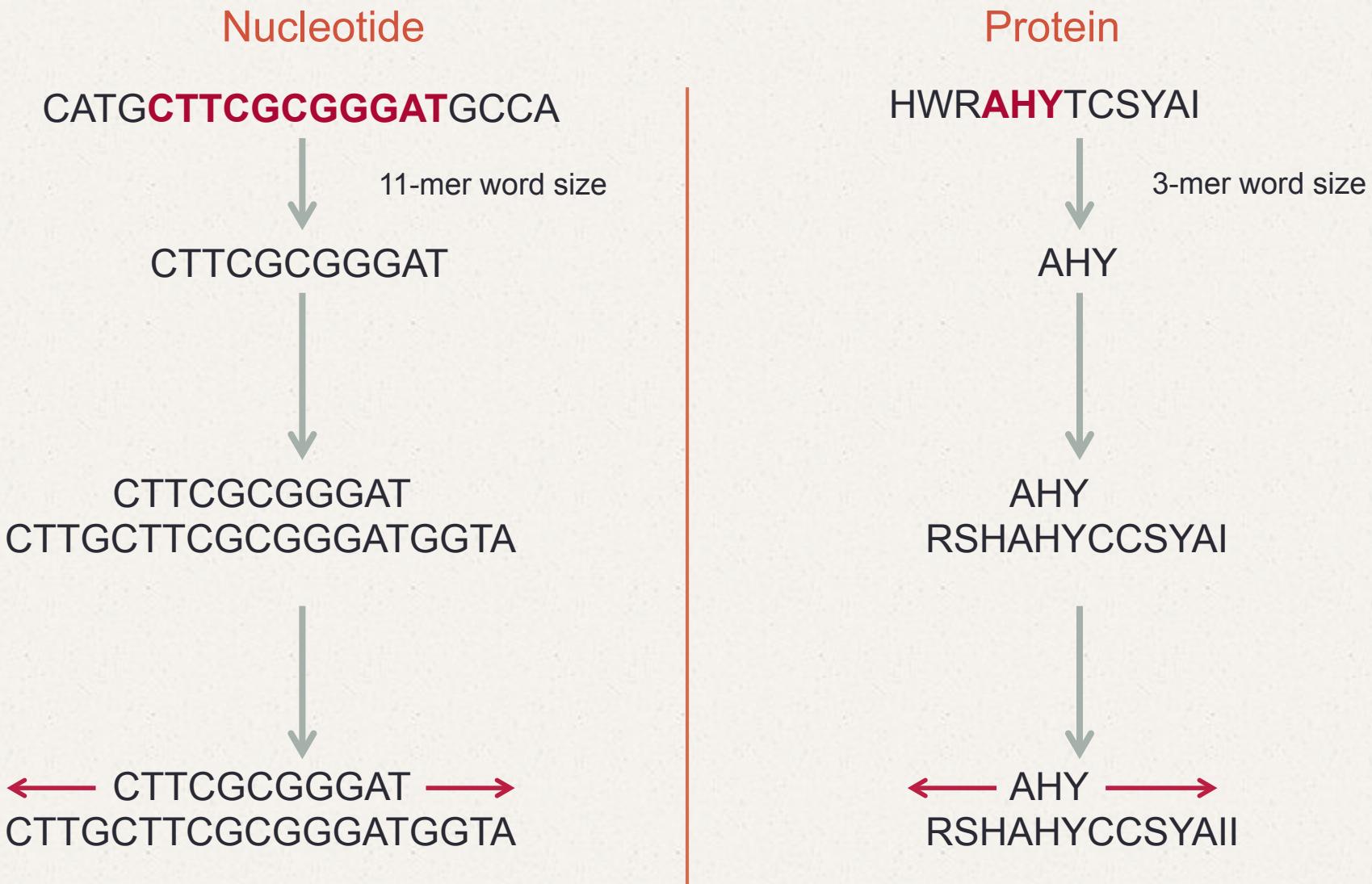
- 11-letter words (seeds)
 - ACTACGTGCTATGC
 - ACTACGTGCTA
 - CTACGTGCTAT
 - TACGTGCTATG
 - ACGTGCTATGC

Protein

- 3-letter words (seeds)
 - PQGDEF
 - PQG
 - QGD
 - GDE
 - DEF



How it works



Blast Scoring and E-values

- Nucleotide sequences search for 11-letter matches
 - $(4^{11} = 4,194,304$ combinations)
 - Match = +5, mismatch = -4
 - Only scores above a threshold (T) are kept

ACTACGTGCTA
ACTACGTGCTA
 $5+5+5+5+5+5+5+5+5+5 = 55$

ACTACG**TG**GCTA
ACAAGA**TGG**TA
 $5+5-4+5-4-4+5+5-4+5+5 = 19$



Blast Scoring and E-values

- Proteins use a BLOSUM62 scoring matrix
 - $20 \times 20 \times 20 = 8,000$ possible 3-letter words
 - All possible amino acid pairs are given a score
 - All combinations above a threshold (T) are kept
 - Minimizes search space

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	
C	9																			C	
S	-1	4																		S	
T	-1	1	5																	T	
P	-3	-1	-1	7																P	
A	0	1	0	-1	4															A	
G	-3	0	-2	-2	0	6														G	
N	-3	1	0	-2	-2	6														N	
D	-3	0	-1	-1	-2	-1	1	6												D	
E	-4	0	-1	-1	-1	-2	0	2	5											E	
Q	-3	0	-1	-1	-1	-2	0	0	2	5										Q	
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	8									H	
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5								R	
K	3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	5							K	
M	-1	-1	-1	-2	-1	-3	-2	-3	0	-2	-1	-1	5							M	
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	1	4						I	
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	4				L	
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	-2	1	3	1	4				V	
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6			F	
Y	-2	-2	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	7			Y	
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	11	W

P Q G

P E G

$$7+2+6 = 15$$

P Q G

E Q R

$$-1+5+-2 = 2$$



Extending Matches

- Match = HSP (High-scoring Sequence Pair)
 - Match is found and extended as long as score stays above a threshold value
 - After finished extending, the HSP is kept if above the cutoff score (S)

Query sequence: R P P Q G L F

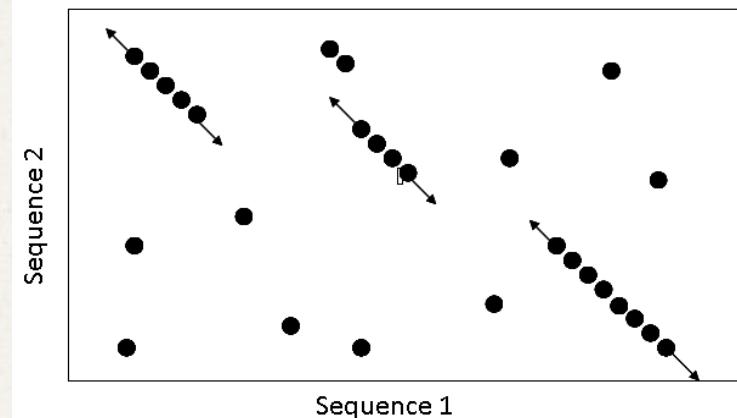
Database sequence: D P P E G V V

Exact match is scanned.

Score: -2 7 7 2 6 1 -1

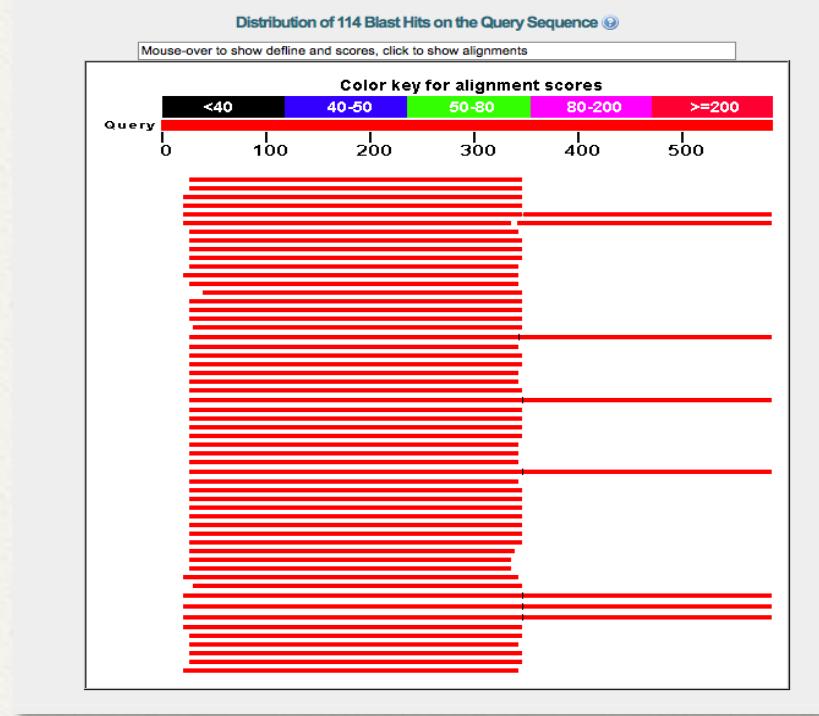
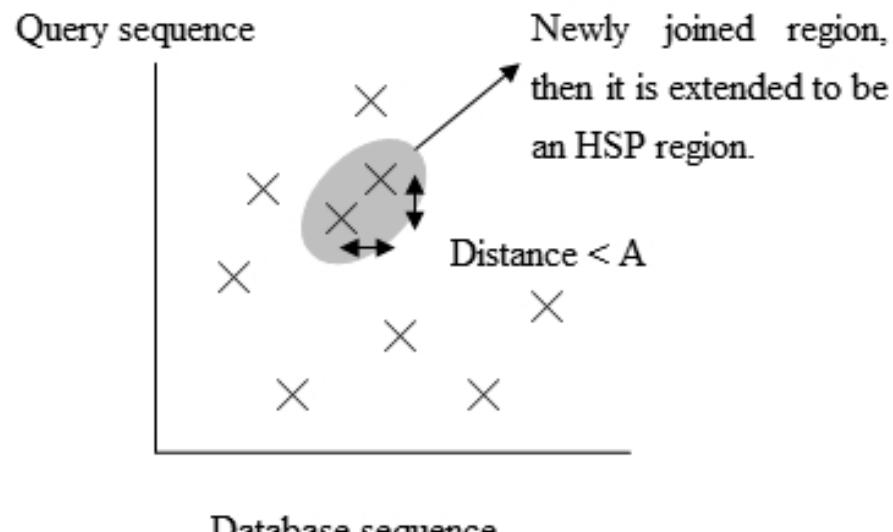
HSP

Optimal accumulated score = $7+7+2+6+1 = 23$



Assembling HSPs

- HSPs, after extension, are assembled into a longer alignment



Output

- Max/Total Score: quality of the alignment
 - Higher the score the better the match
- Query Coverage: what proportion of the query the particular HSP covers
- E-value: probability that a match \geq Max Score occurs by random chance (based on database size)
- Max Identity: For that HSP, the % of bases that match

Accession	Total Score	Query Coverage	E-value	Max Ident
X56286.1	579	54%	7e-162	99%
AF091629.1	573	54%	3e-160	99%
L48348.1	481	55%	2e-132	93%



Interpretation

- The matches you get are only acceptable matches, not necessarily the optimal match
- Your search is only as good as your database
 - If the optimal match is not in the database, you will not find it.
 - If you have sequences not in the database, SUBMIT THEM!

Pandyland.net



Take Home Points

- Blast is a powerful tool for database searching
- Very fast, but at the expense of sensitivity
- Flexible (types, databases)
- Interpret results carefully
- Help make it grow!



Several examples

- Example 1: SRA Blast (<https://www.ncbi.nlm.nih.gov/sra>)
 - Query: M55627.1
 - *Coccidioides immitis* (Valley fever fungus) ssuRNA
 - Project: SRX633288
 - Puma 454 transcriptome reads
- Example 2: Blast a genome (<https://blast.ncbi.nlm.nih.gov/>)
 - Query: KU740200.1
 - MERS virus genome
 - Database: nt (Select genus *Camelus* – 9836) (blastn)



Lunch!



"Mr. Osborne, may I be excused? My brain is full."

