BLAST

Basic Local Alignment Search Tool

So useful – it is now a verb in the literature

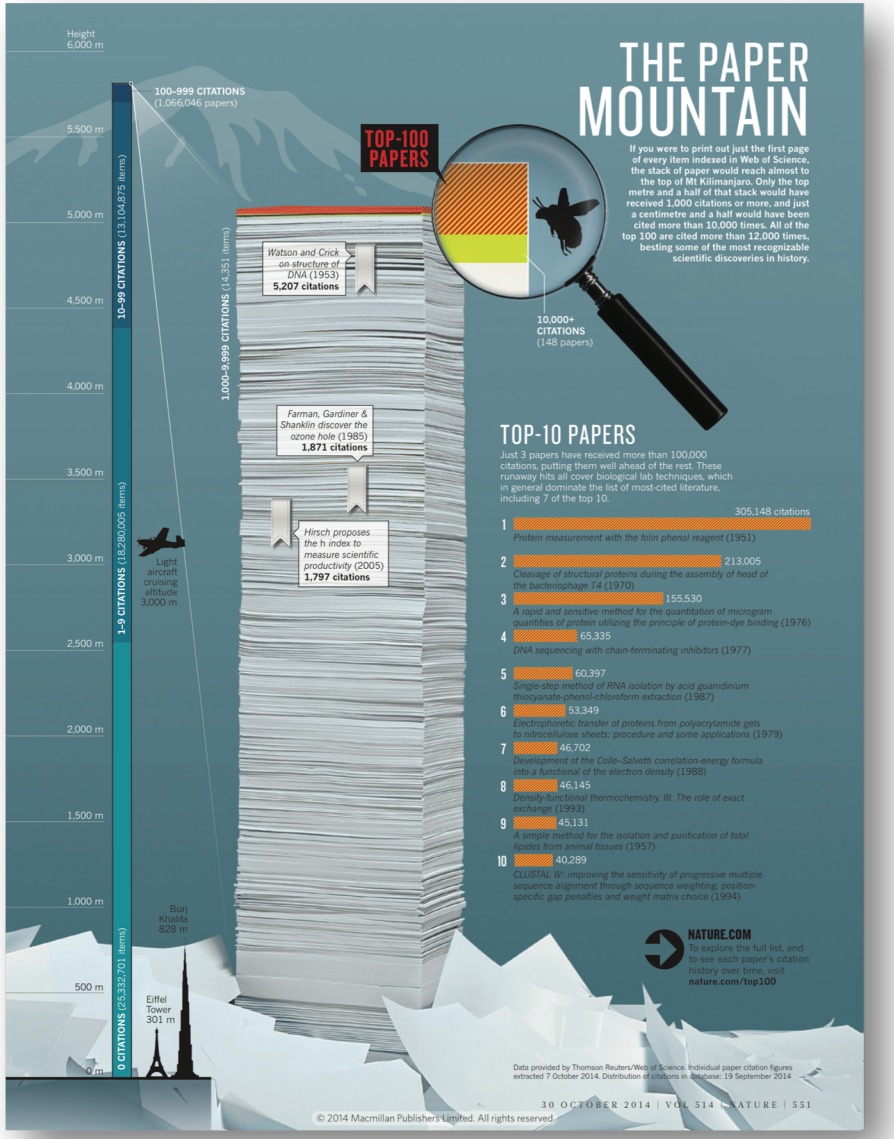
Goals

- What is BLAST and why is it important?
- Principles of the algorithm
- Online Examples
- Command Line Implementation

A Lot of BLASTing

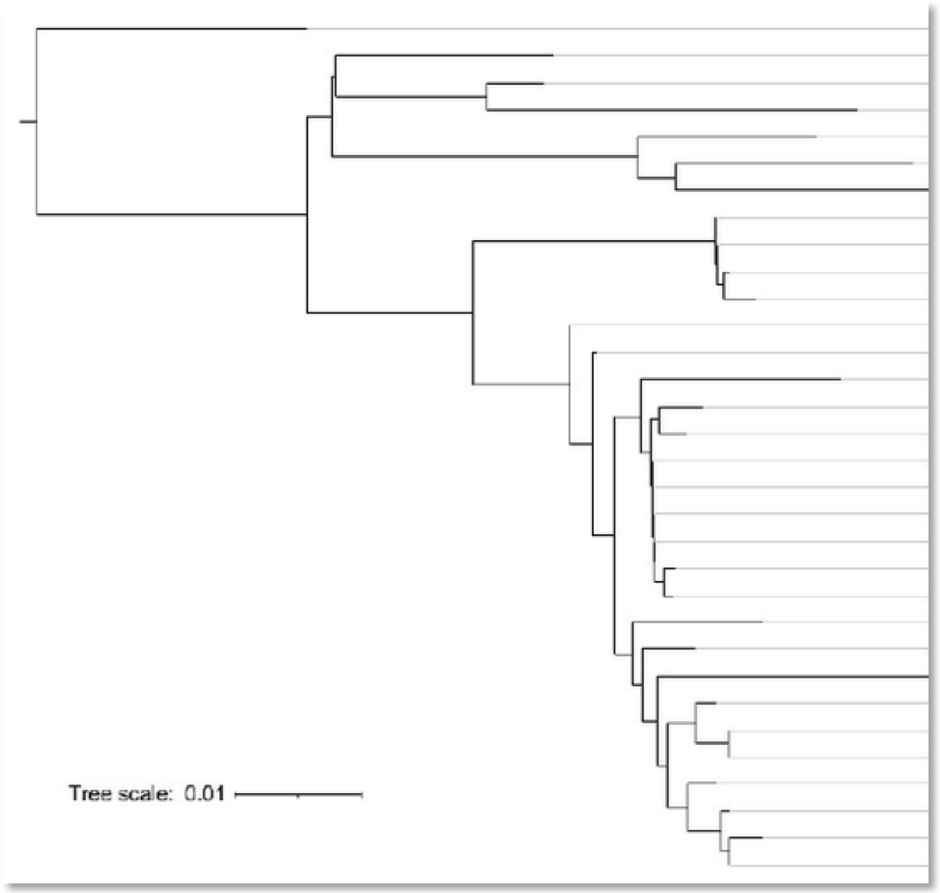
- Where is BLAST on this list?
  - Altschul et al. 1990
    - #12 – 38,380 citations
      - 62,483 (Web of Science 8/1/2021) - #10
  - Altschul et al. 1997
    - #14 – 36,410 citations
      - 53,647 (Web of Science 8/1/2021) - #14
  - Combined: 4th!

Van Noorden et al. 2014, *Nature*



BLAST

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



BLAST

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

- Global vs Local Alignment
  - Global alignment: entire sequences

  - Local alignment: segments of sequences

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

BLAST Flavors

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)

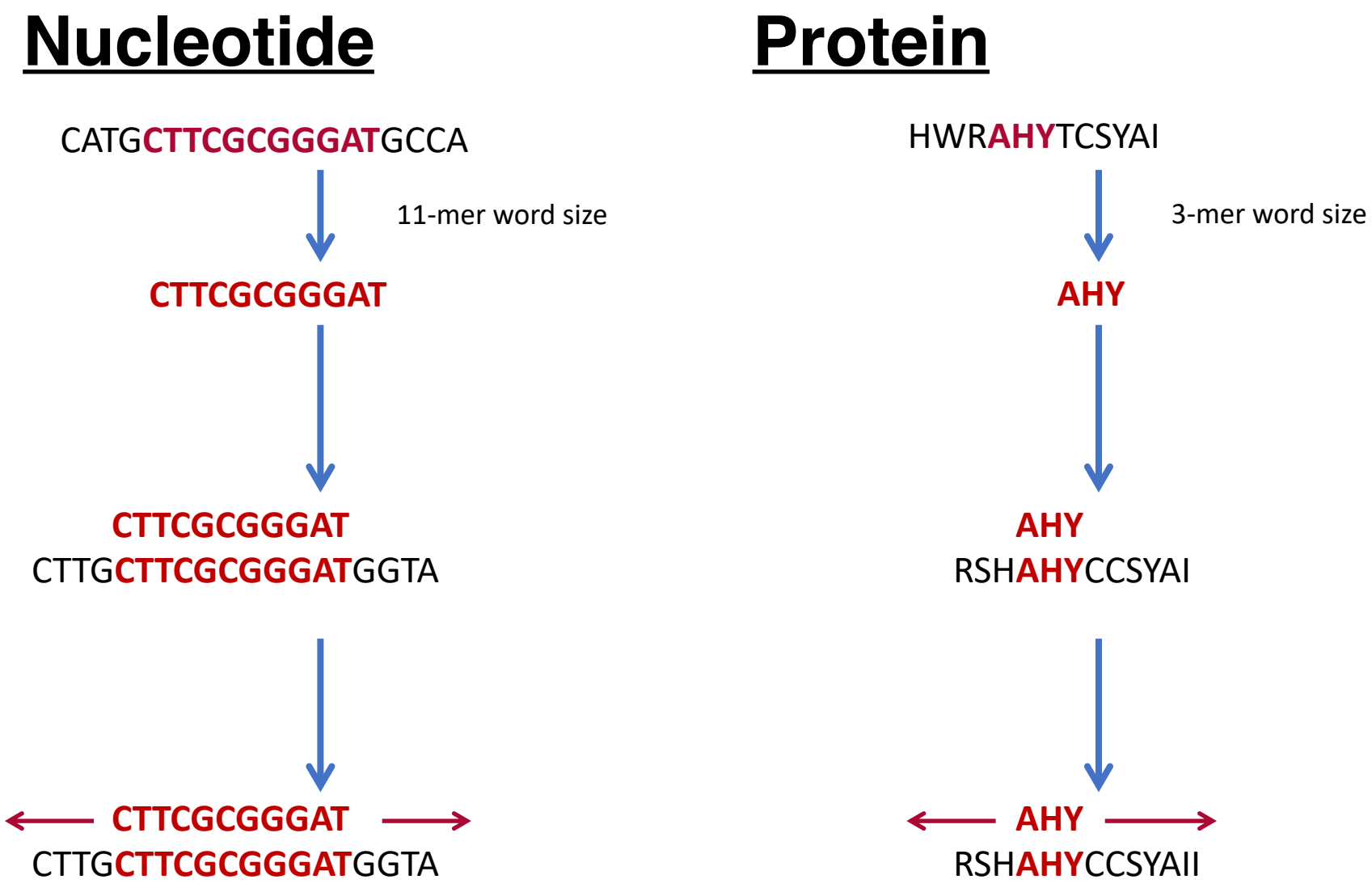
ACTACGTGCTATGC
ACTACGTGCTA
CTACGTGCTAT
TACGTGCTATG
ACGTGCTATGC

Protein

3-letter words (seeds)

PQGDEF
PQG
QGD
GDE
DEF

How it Works


Nucleotide
CATGCTTCGCGGGATGCCA
11-mer word size
CTTCGCGGGAT
CTTCGCGGGAT
CTTGCTTCGCGGGATGGTA
CTTCGCGGGAT
CTTGCTTCGCGGGATGGTA
Protein
HWRAHYTCSYAI
3-mer word size
AHY
AHY
RSHAHYCCSYAI
AHY
RSHAHYCCSYAII

BLAST Scoring and E-values

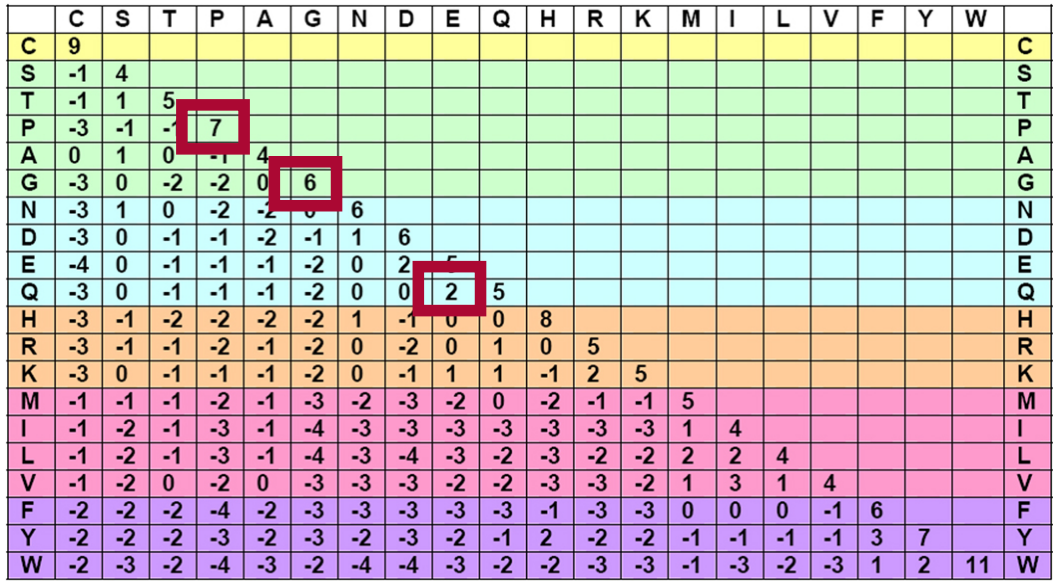
- Nucleotide sequences search for 11-letter matches
  - 4^11 = 4,194,304 combinations
  - Match = +5, mismatch = -4
  - Only scores above a threshold (T) are kept

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

ACTACGTGCTA
ACAAGATGGTA
5+5-4+5-4-4+5+5-4+5+5 = 19

BLAST Scoring and E-values

- Proteins use a BLOSUM62 scoring matrix
  - 20 x 20 x 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



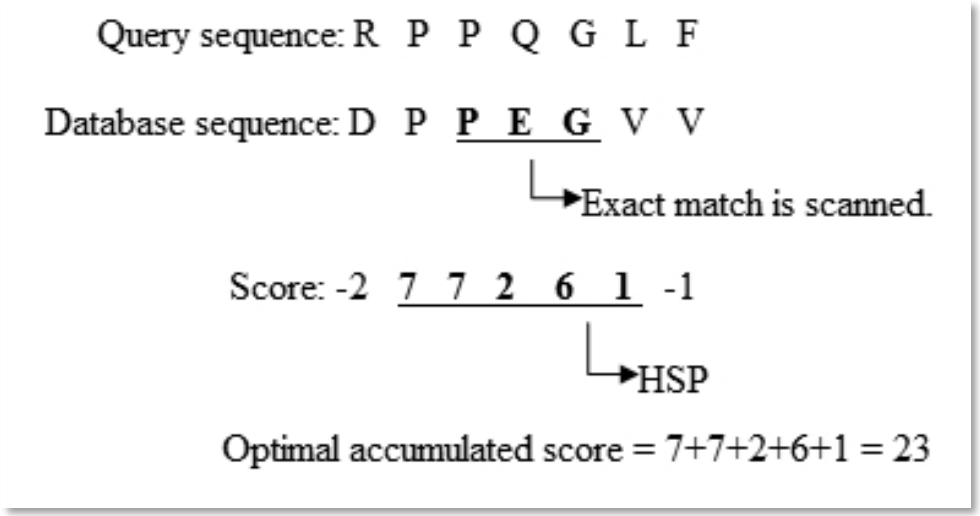
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	
C	9																				C
S	-1	4																			S
T	-1	1	5																		T
P	-3	-1	-1	7																	P
A	0	1	0	-1	4																A
G	-3	0	-2	-2	0	6															G
N	-3	1	0	-2	-2	0	6														N
D	-3	0	-1	-1	-2	-1	1	6													D
E	-4	0	-1	-1	-1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

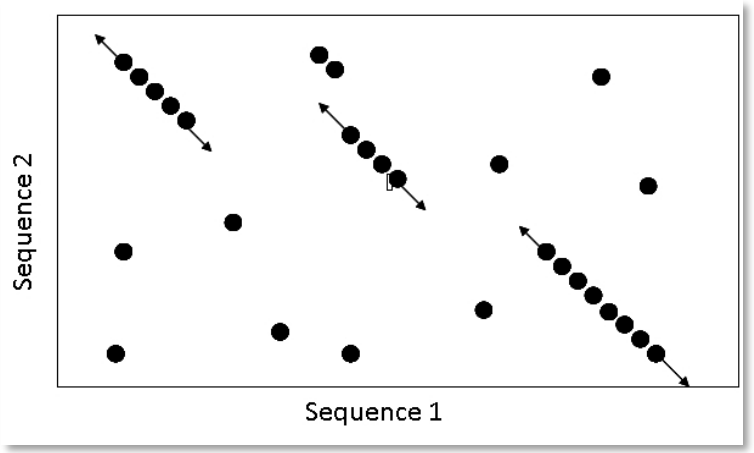
P Q G
P E G
7+2+6 = 15

P Q G
E Q R
-1+5+-2 = 2

Extending Matches

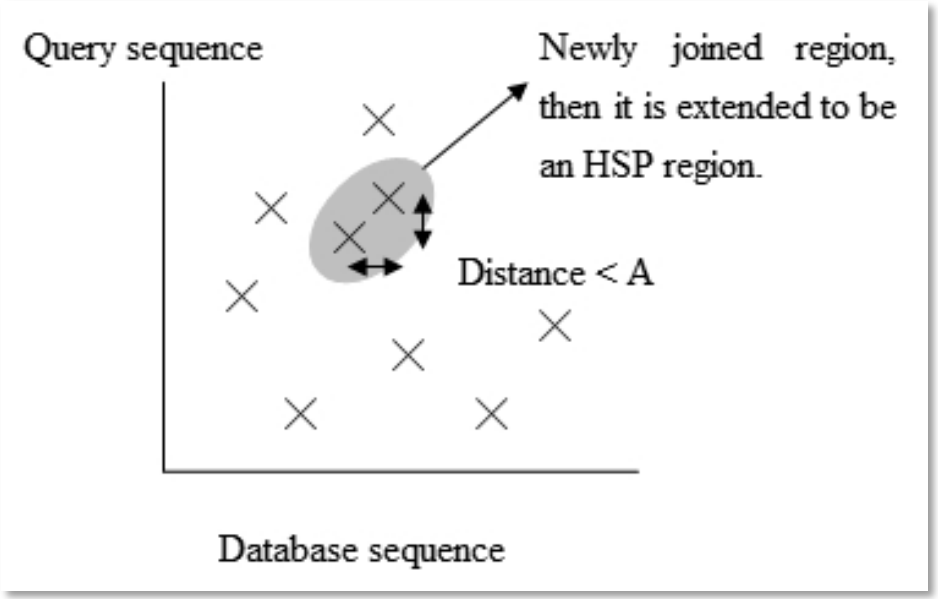
- Match = HSP (High-scoring Sequence Pair)
  - Match is found and extended as long as score stays above a threshold value
    - After finished extending, the HSP is kept if above the cutoff score (S)

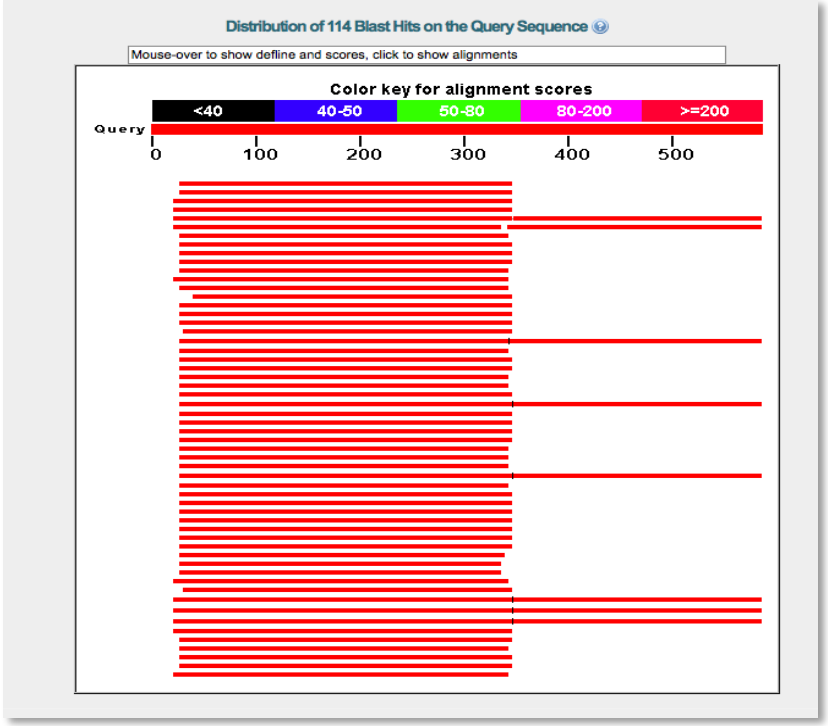




Assembling HSPs

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





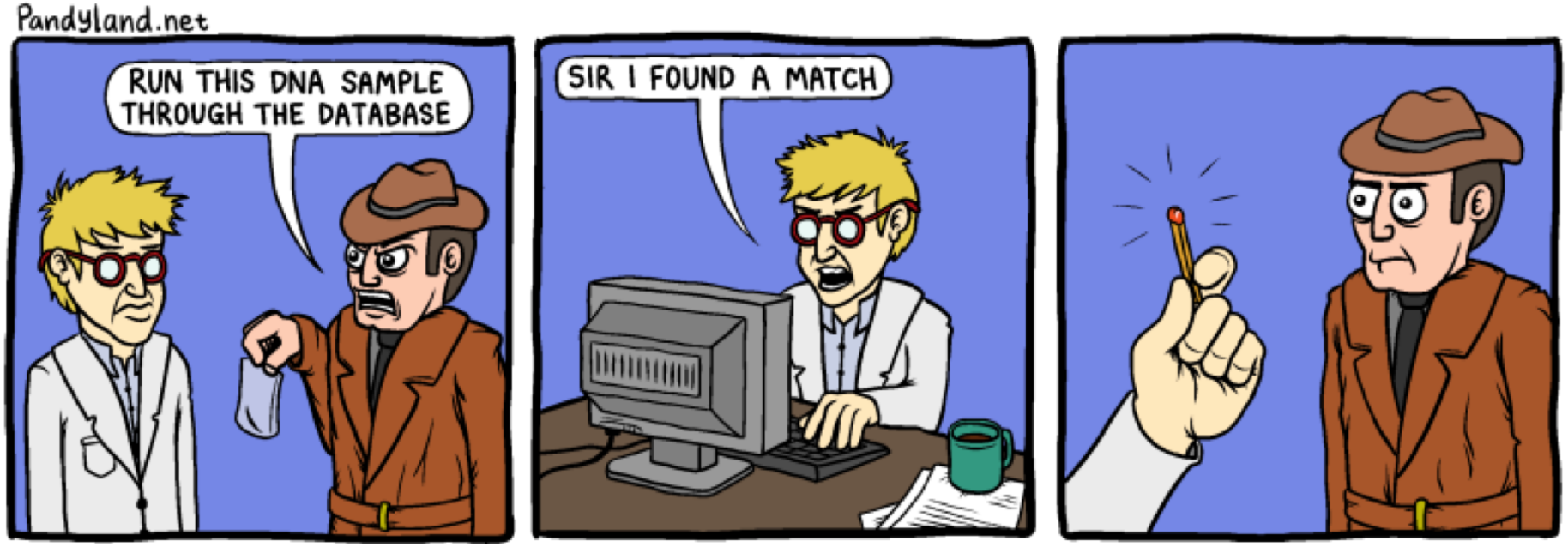
Output

- 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 ≥ 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!



Take Away Points

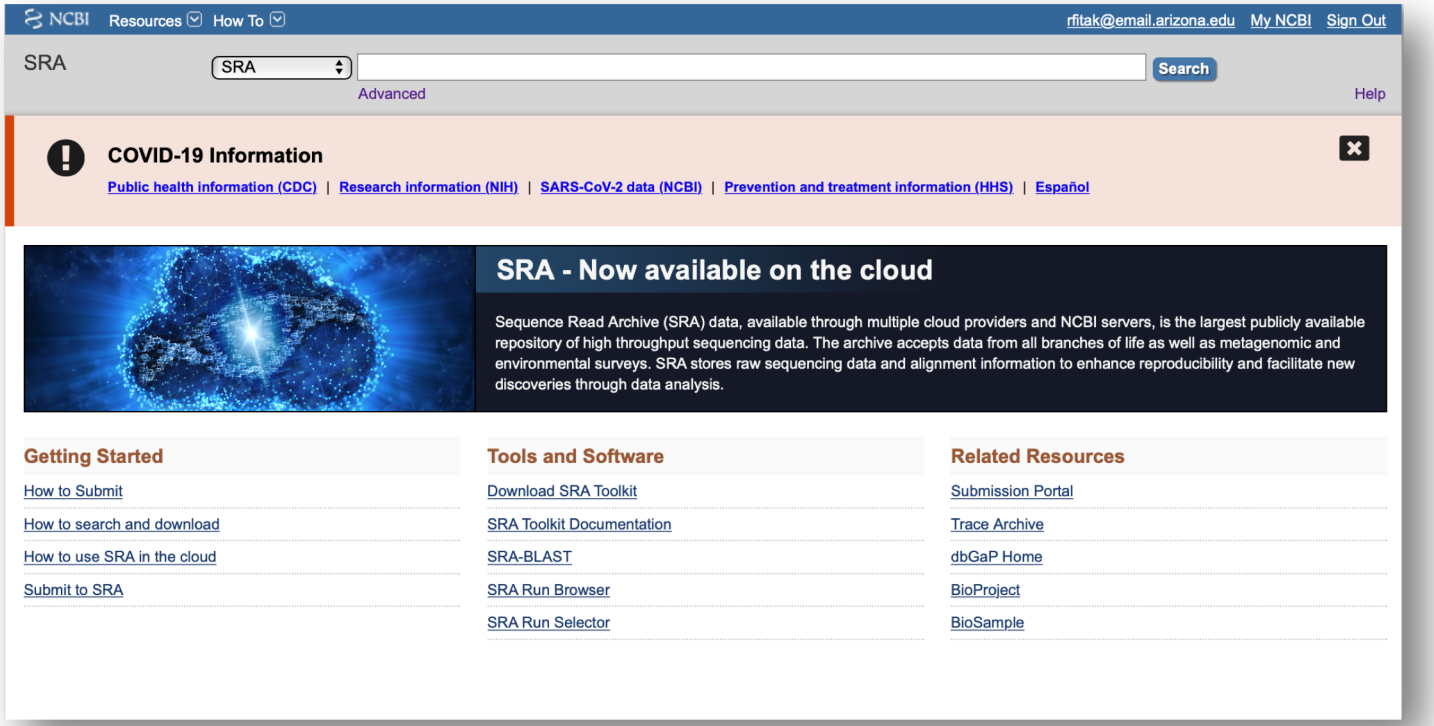
- BLAST is a powerful tool for database searching
- Very fast, but at the expense of sensitivity
- Flexible (types, databases)
- Interpret results carefully
- Help make it grow!

DATABASES

Where are the genomic data?

SRA

- **S**equence **R**ead **A**rchive
  - https://www.ncbi.nlm.nih.gov/sra/

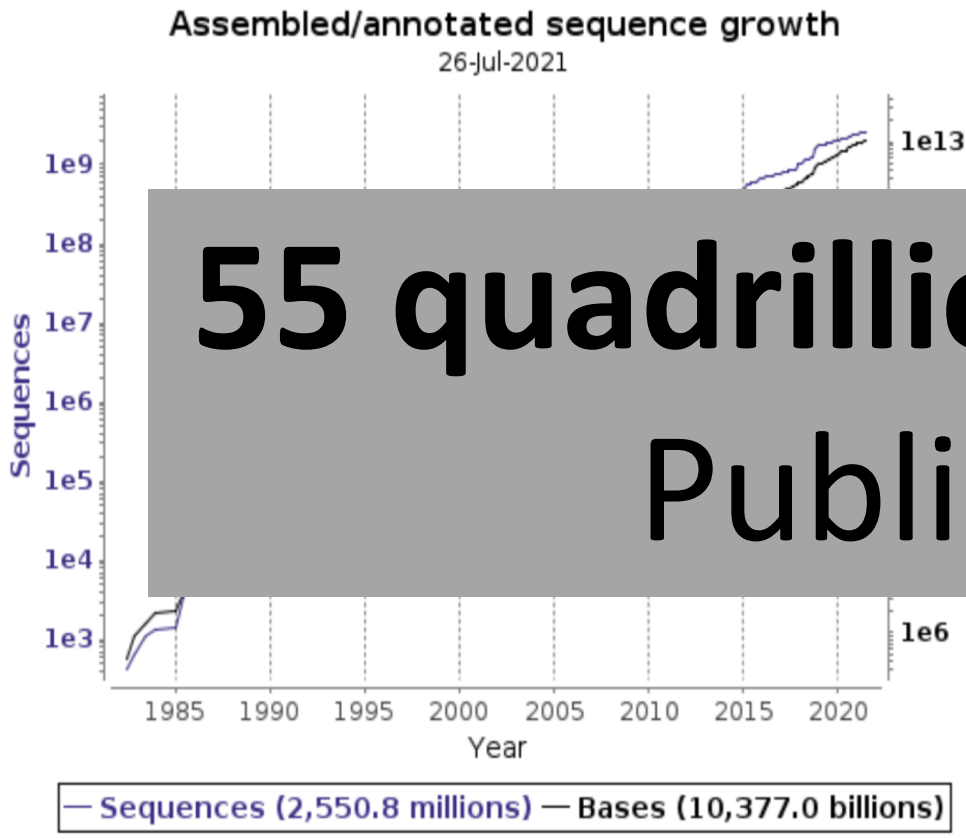


So how big is it?

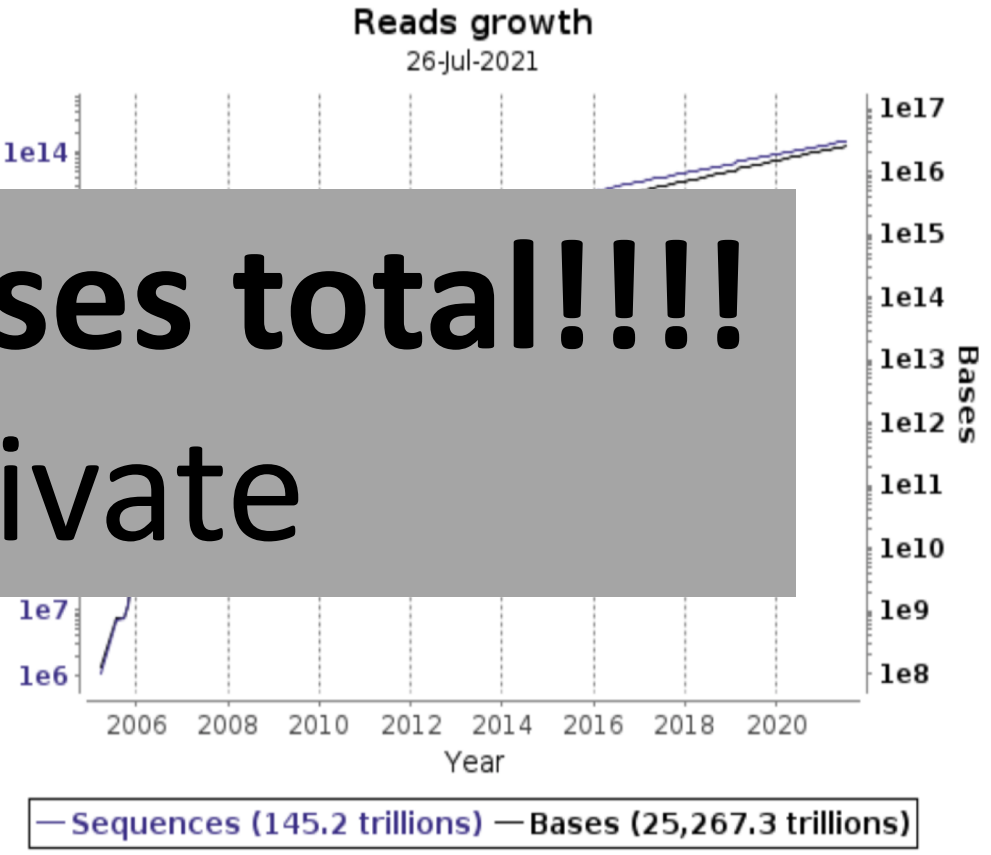
Guesses?

DNA Sequence Databases (GenBank, SRA, ENA)

Assembled/annotated sequence growth



Reads growth



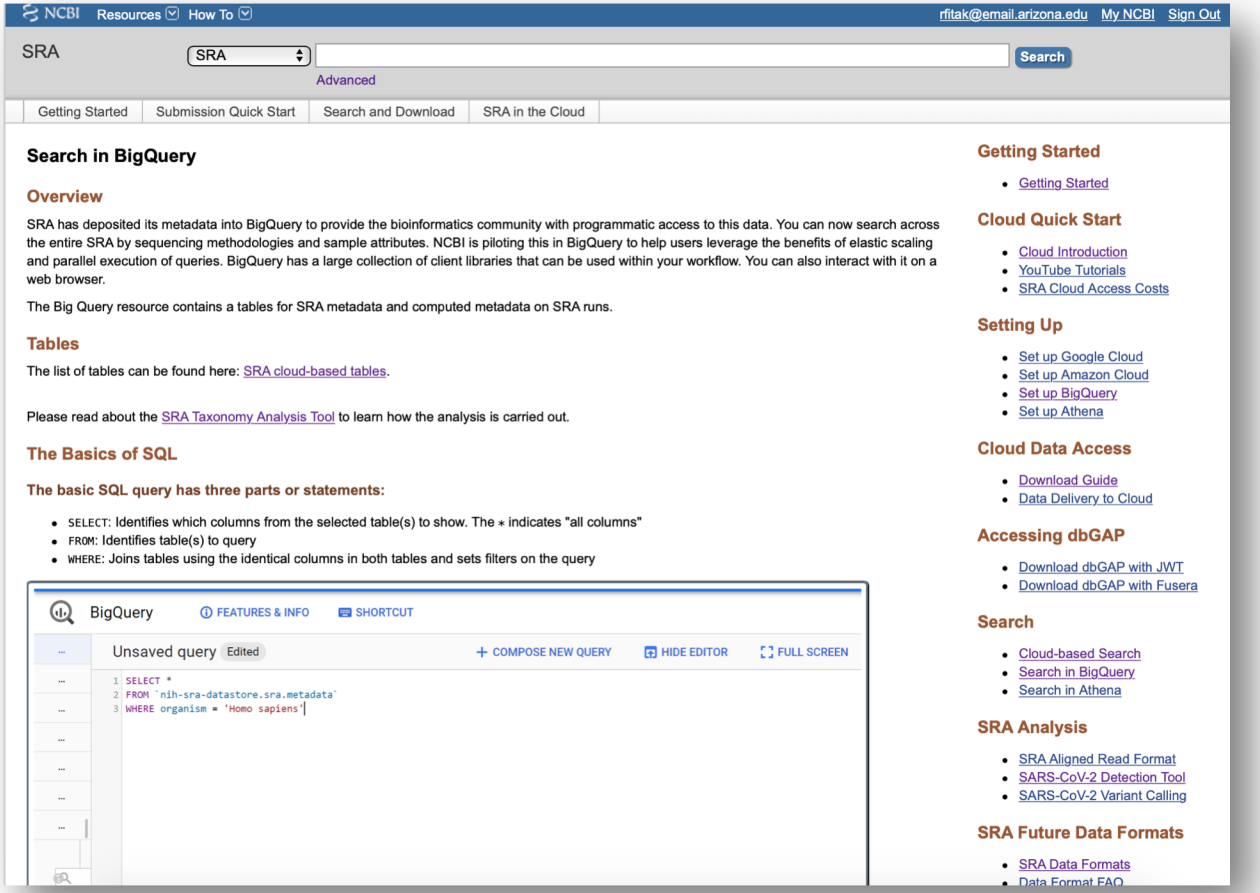
55 quadrillion bases total!!!!

Public + Private

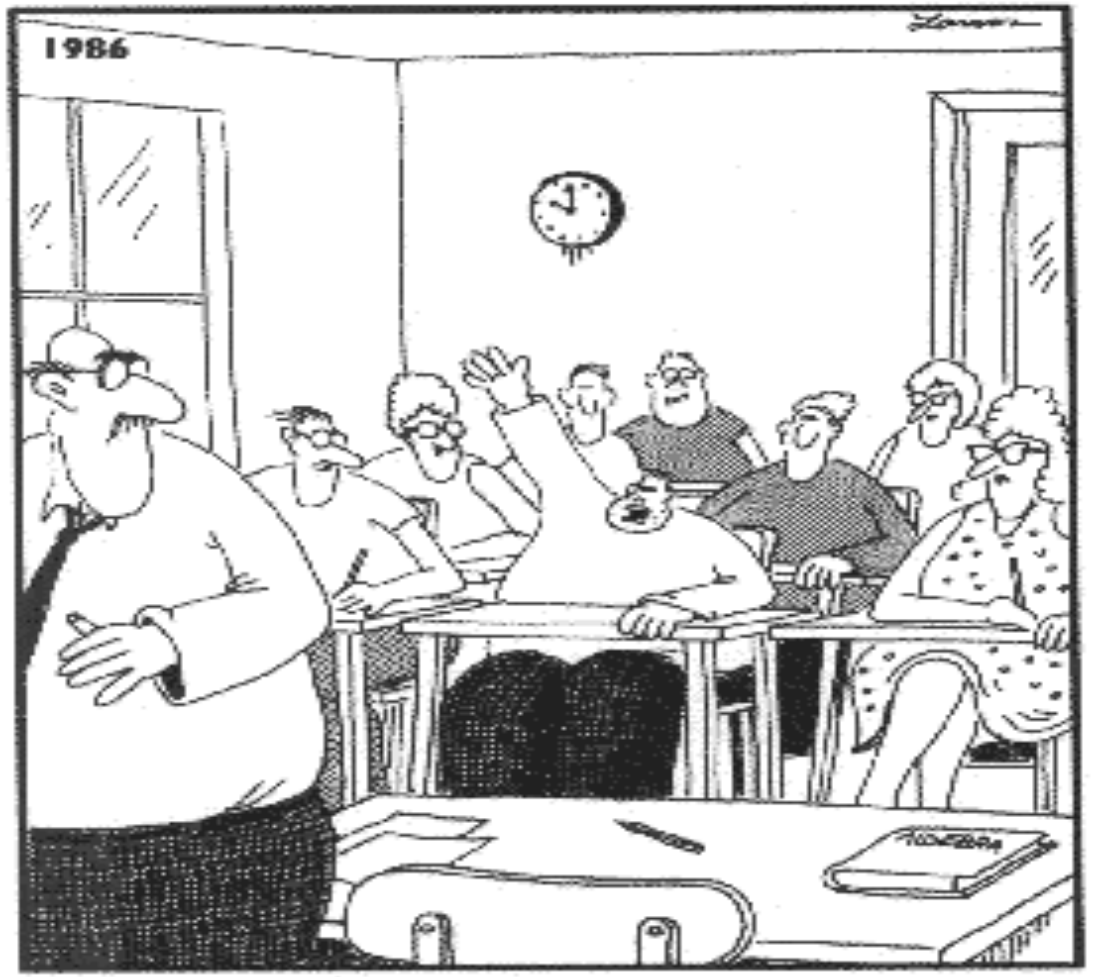
https://www.ebi.ac.uk/ena/browser/about/statistics

SRA Demo…

How to search all that SRA data?



BigQuery



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

Practice Examples

- Example 1: SRA Blast (https://www.ncbi.nlm.nih.gov/sra)
  - Click "**SRA-BLAST**" link
  - Query: M55627.1
    - *Coccidiodes immitis* (Valley fever fungus) ssuRNA
  - Project: SRX633288
    - Puma 454 transcriptome reads

- Example 2: Blast an assembly (https://blast.ncbi.nlm.nih.gov/)
  - Select "**Nucleotide BLAST**"
  - Query:
    - TruSeq Universal Adapter
    - AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
  - Database: nt
  - Organism: *Cyprinus carpio (taxid:7962)*