Who Am I?

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BLAST

Basic Local Alignment Search Tool

So useful – it is now a verb in the literature

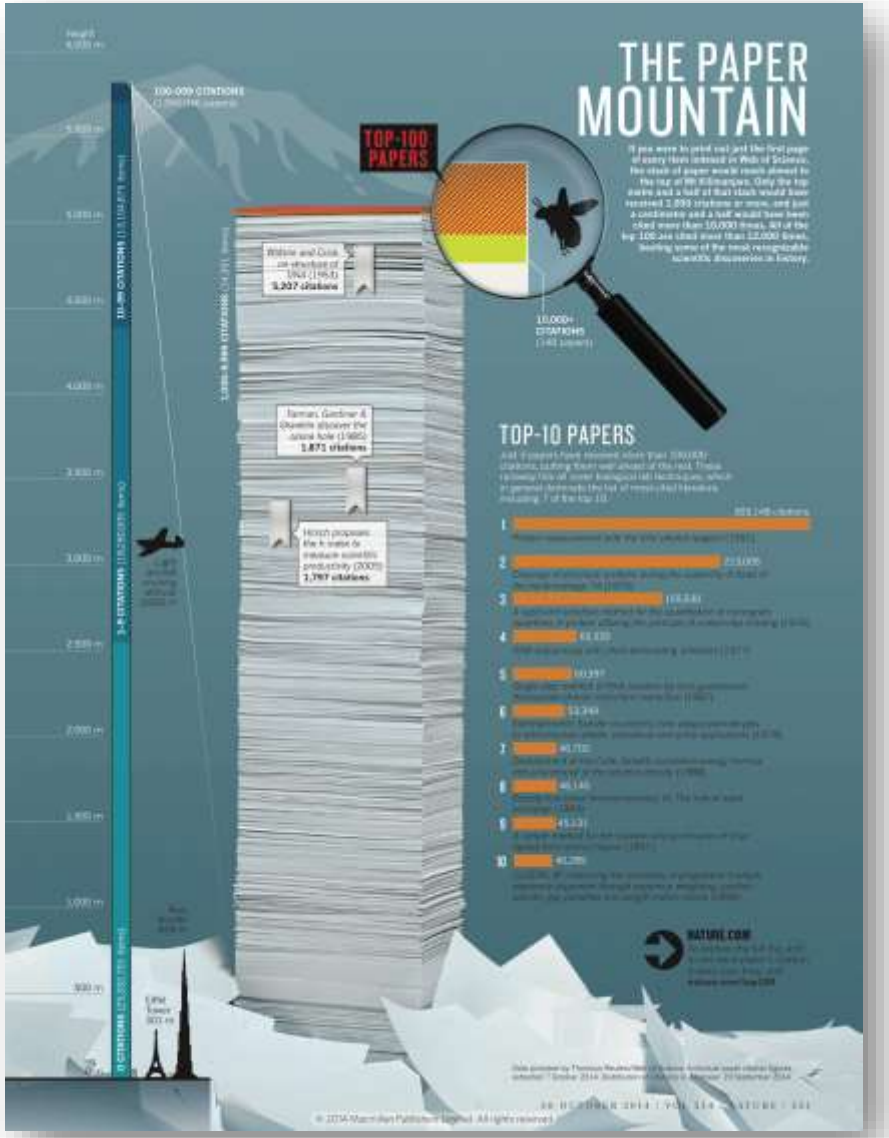
Goals

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

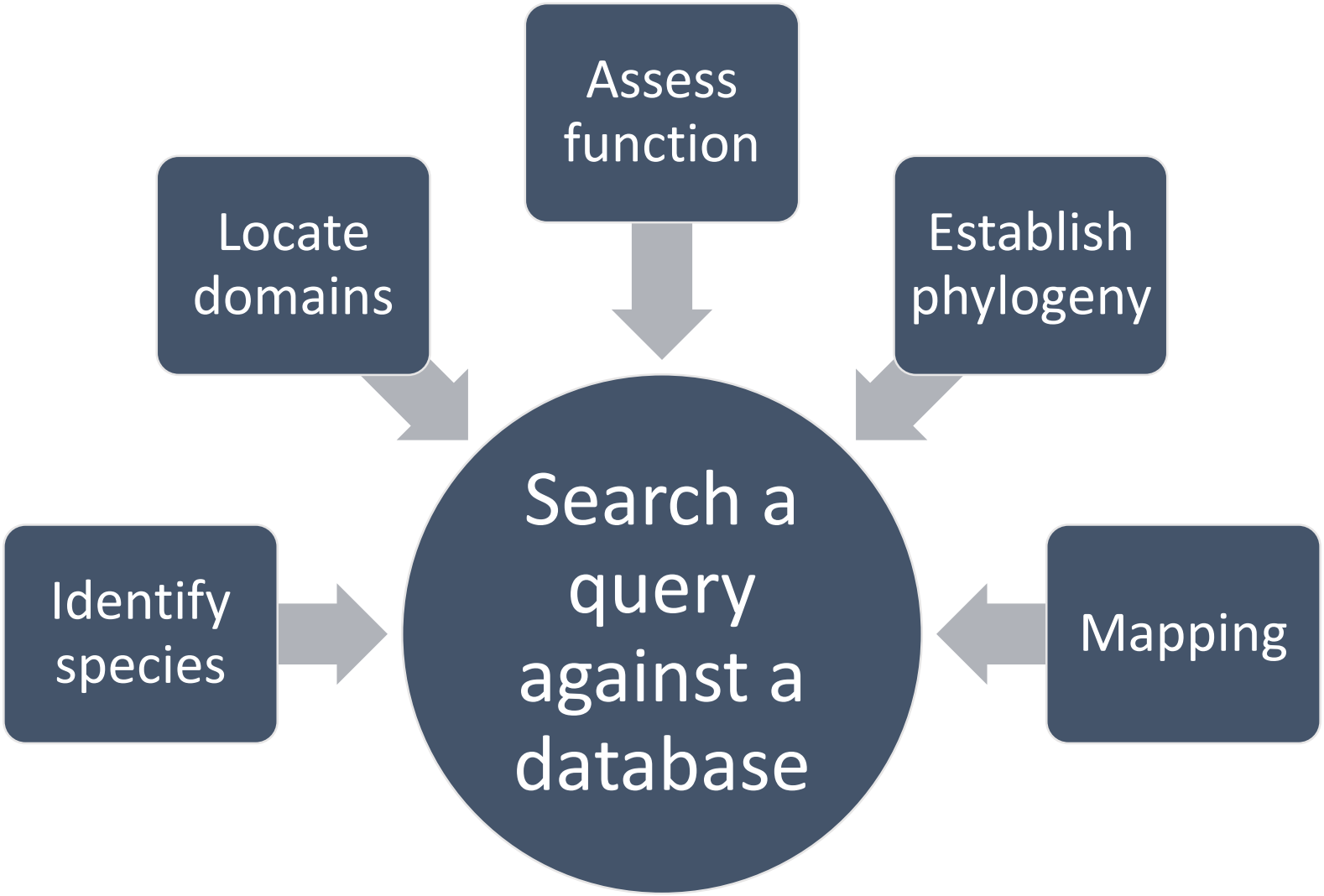
A Lot of BLASTing

- Where is BLAST on this list?
  - Altschul et al. 1990
    - #12 – 38,380 citations
      - 69,412 (Web of Science 6/4/2023) - #10
  - Altschul et al. 1997
    - #14 – 36,410 citations
      - 57,026 (Web of Science 6/4/2023) - #14
  - Combined: 4th!

Van Noorden et al. 2014, *Nature*



BLAST


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

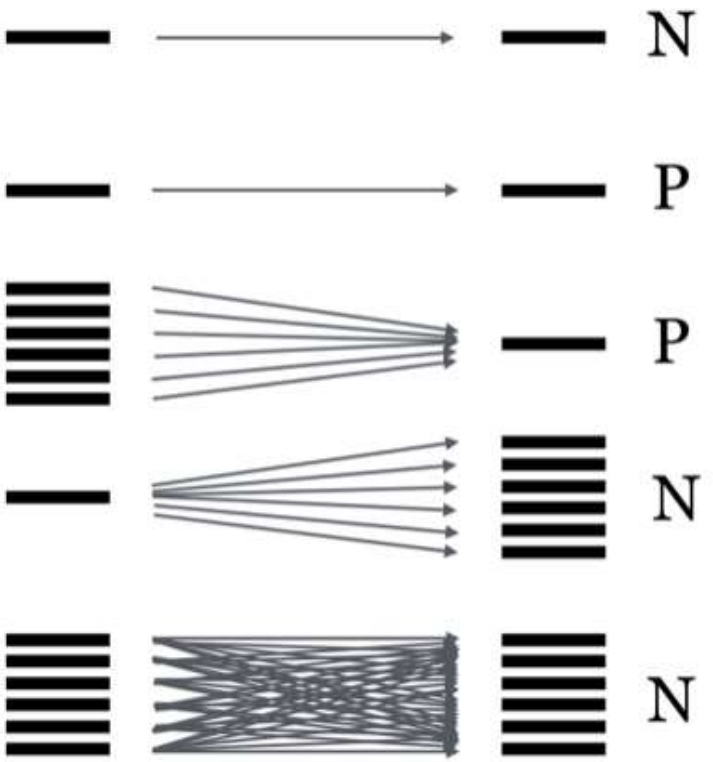
  - Local alignment often the most relevant
    - Depends on biological assumptions

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 Flavors

Program	Query Type	Subject Type	Computation
blastn	N	N	~ 1X
blastp	P	P	~ 1X
blastx	N	P	~ 6X
tblastn	P	N	~ 6X
tblastx	N	N	~36X



(other BLAST types not listed: `psiblast`, `deltablast`, `rpsblast`)

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

CATG**CTTCGCGGGAT**GCCA

11-mer word size

CTTCGCGGGAT

CTTCGCGGGAT
CTTG**CTTCGCGGGAT**GGTA

CTTCGCGGGAT
CTTG**CTTCGCGGGAT**GGTA

Protein

HWR**AHY**TCSYAI

3-mer word size

AHY

AHY
RSH**AHY**CCSYAI

AHY
RSH**AHY**CCSYAII

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)

Query sequence: R P P Q G L F

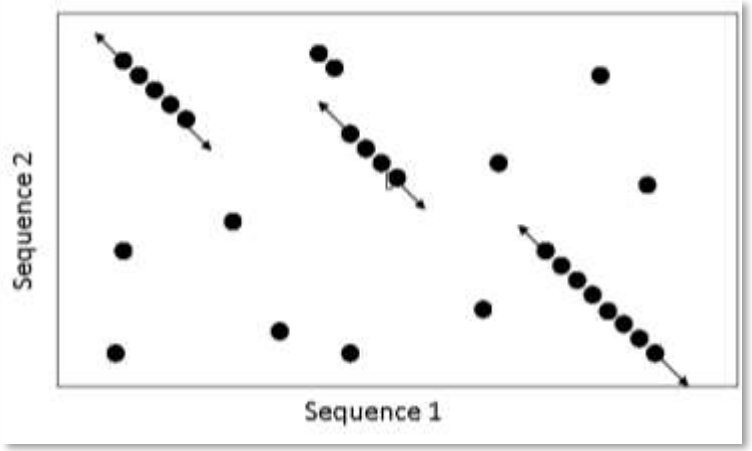
Database sequence: D P **P E G** V V

Exact match is scanned.

Score: -2 **7 7 2 6 1** -1

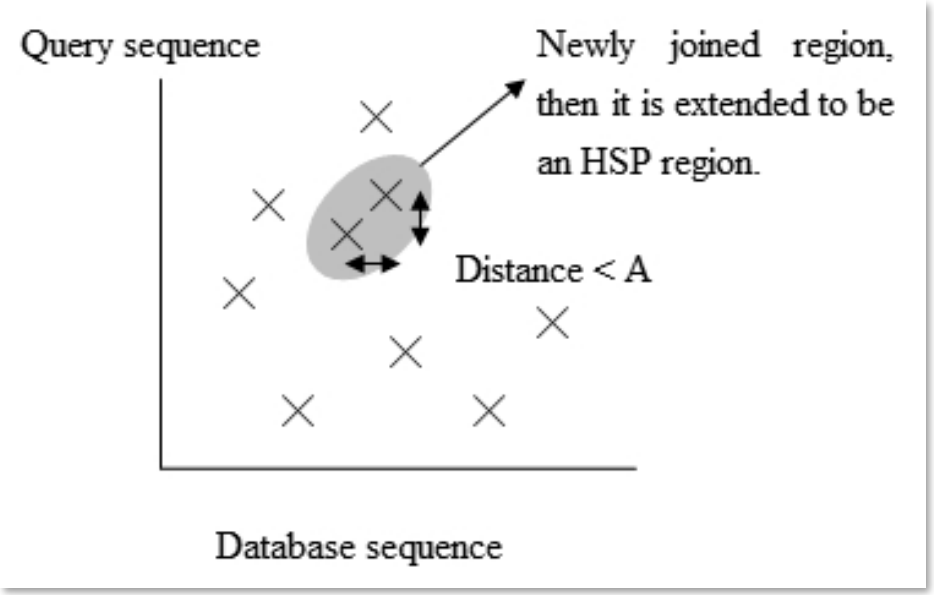
HSP

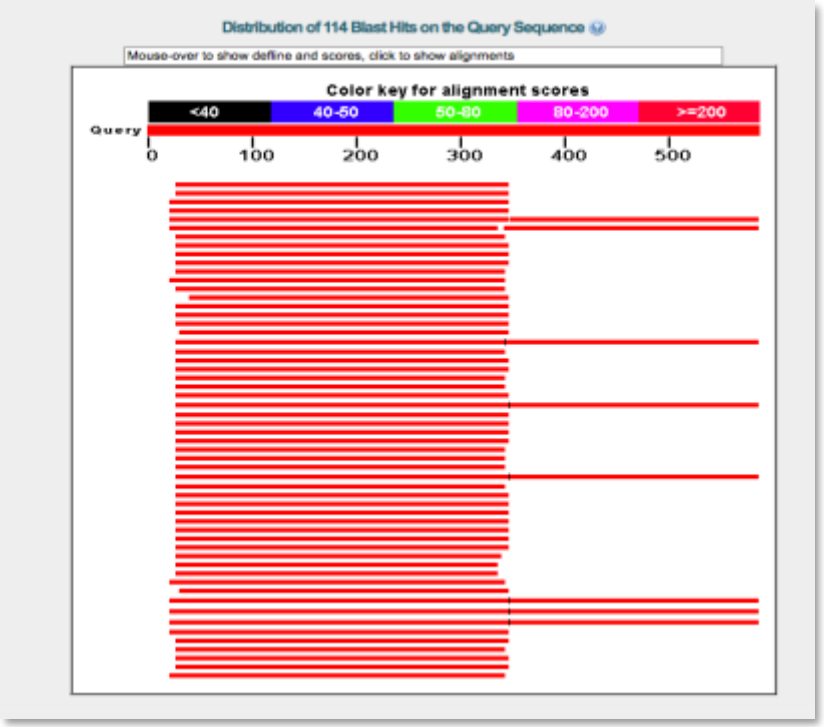
Optimal accumulated score = 7+7+2+6+1 = 23



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 ≥ 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

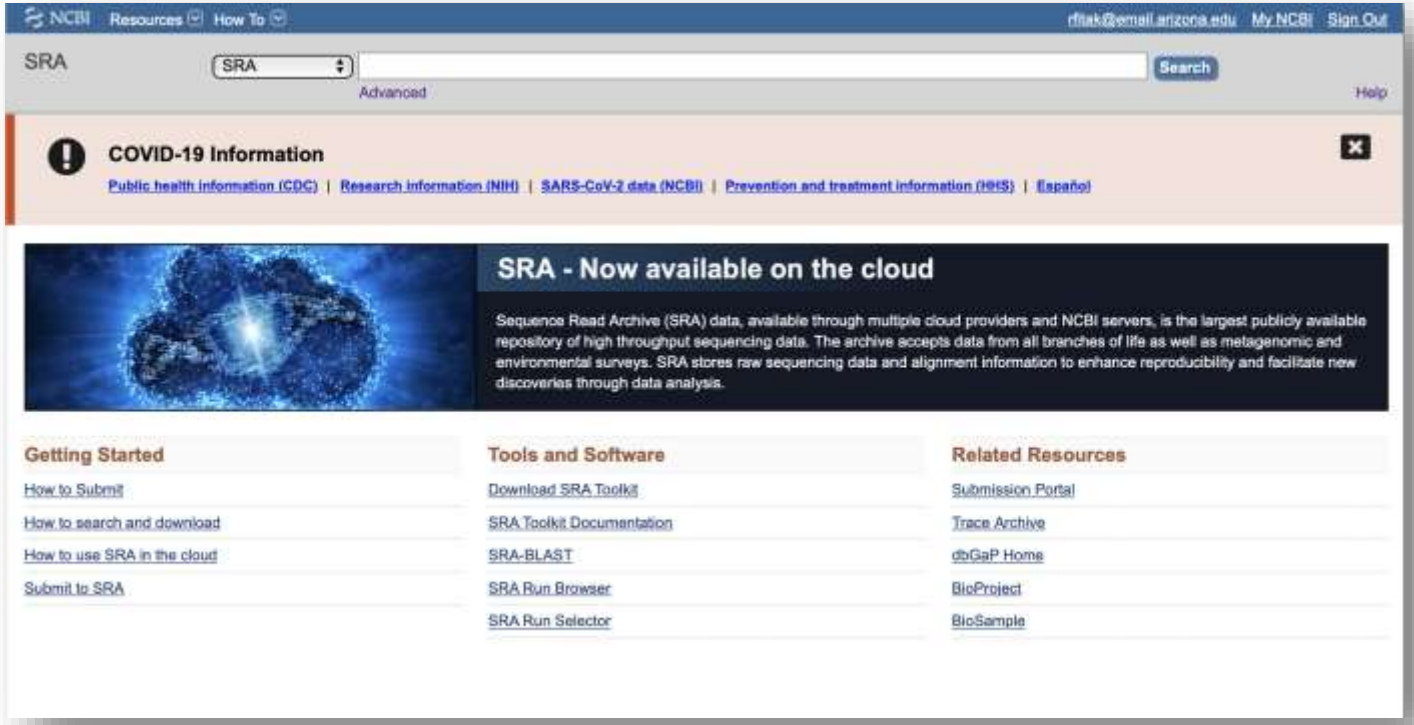
Help make it grow!

DATABASES

Where are the genomic data?

SRA

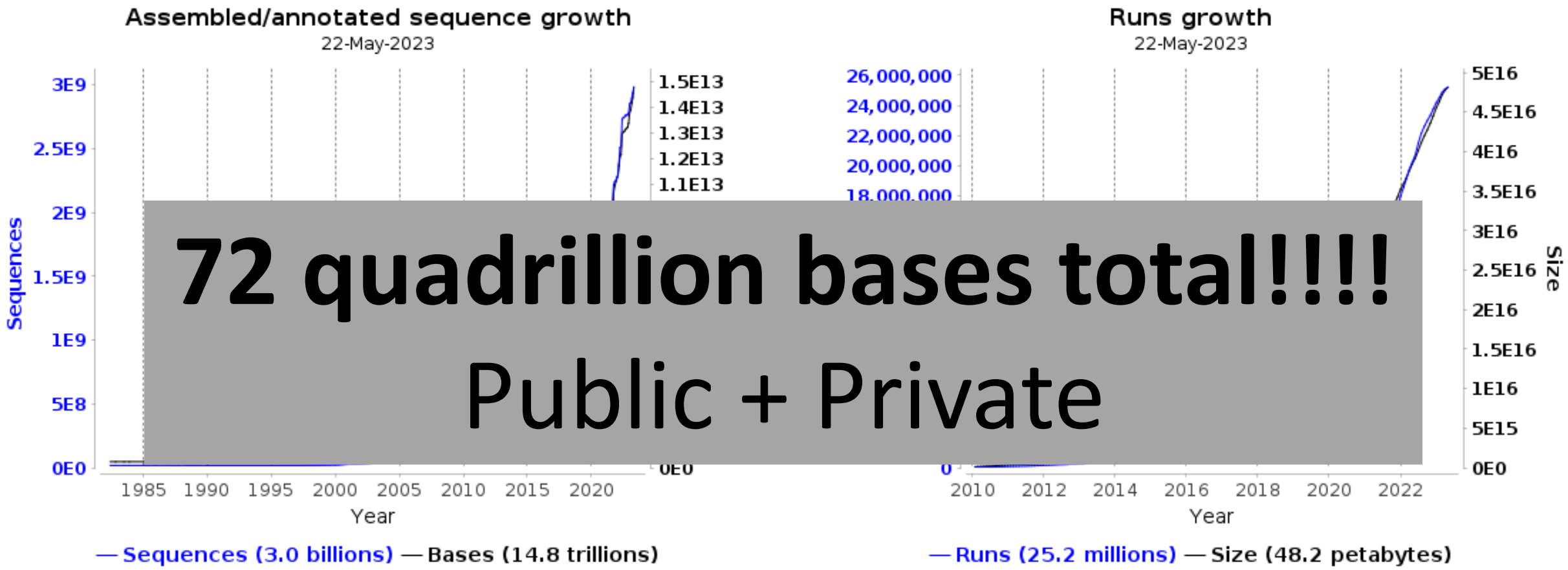
- **S**equence **R**ead **A**rchive
  - https://www.ncbi.nlm.nih.gov/sra/



So how big is it?

Guesses?

DNA Sequence Databases (GenBank, SRA, ENA)



72 quadrillion bases total!!!!

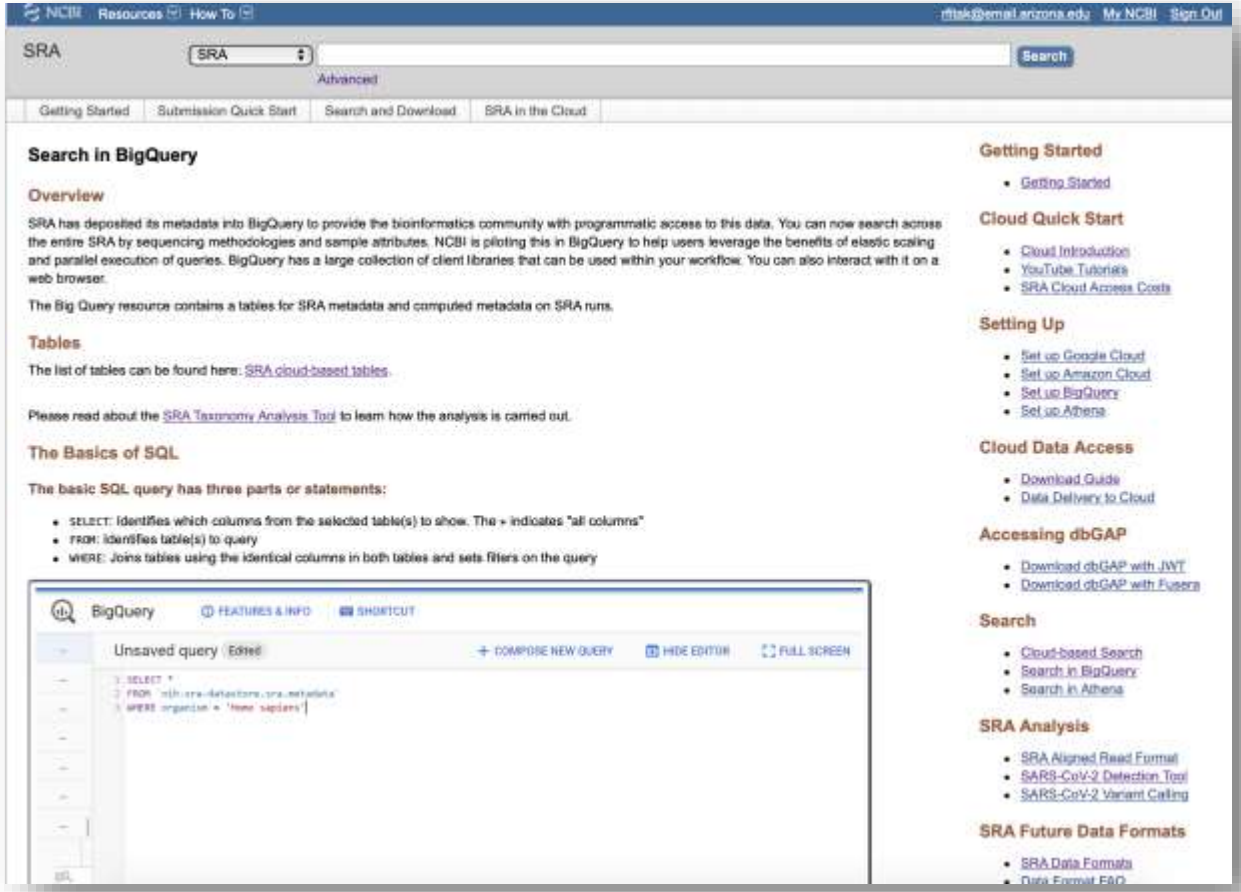
Public + Private

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

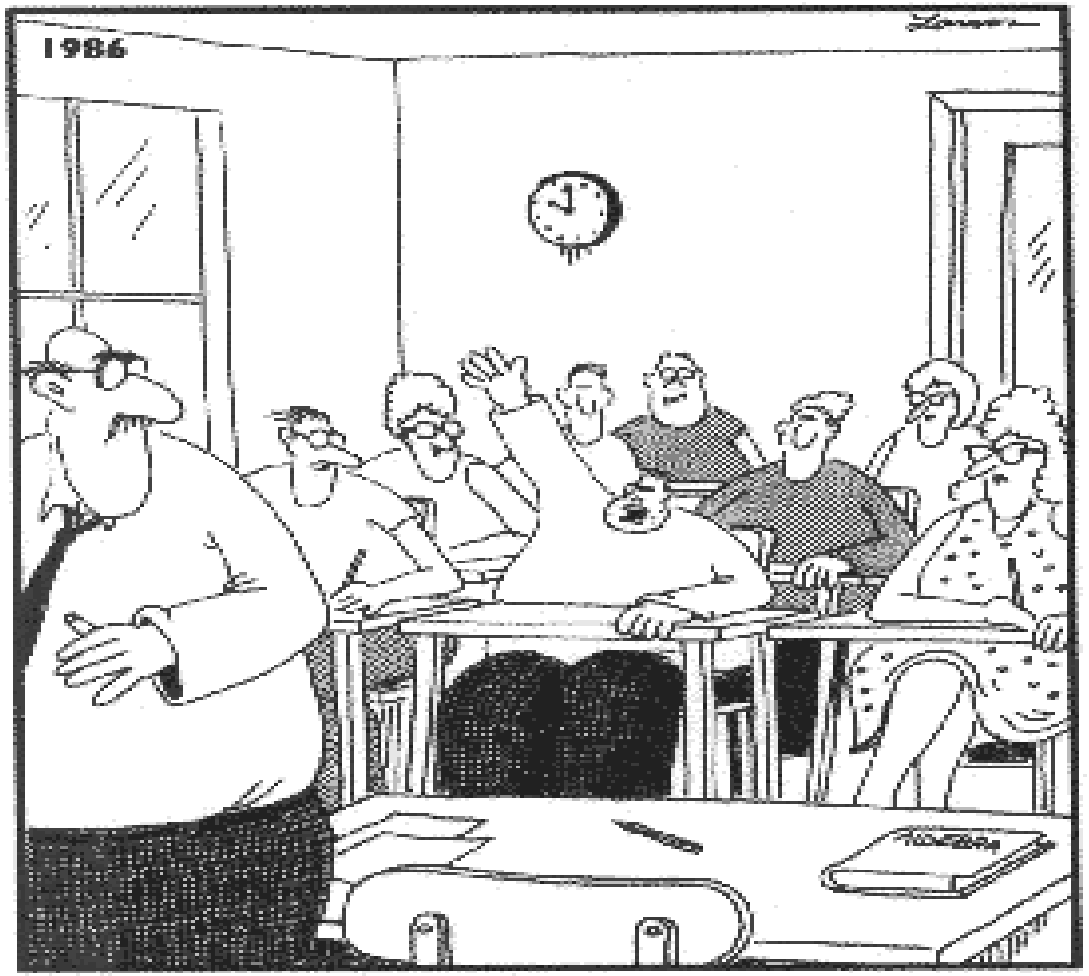
SRA Demo…

1. BioProject PRJEB14687
   https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJEB14687
2. Search SRA for “Fitak”
   https://www.ncbi.nlm.nih.gov/sra

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)*