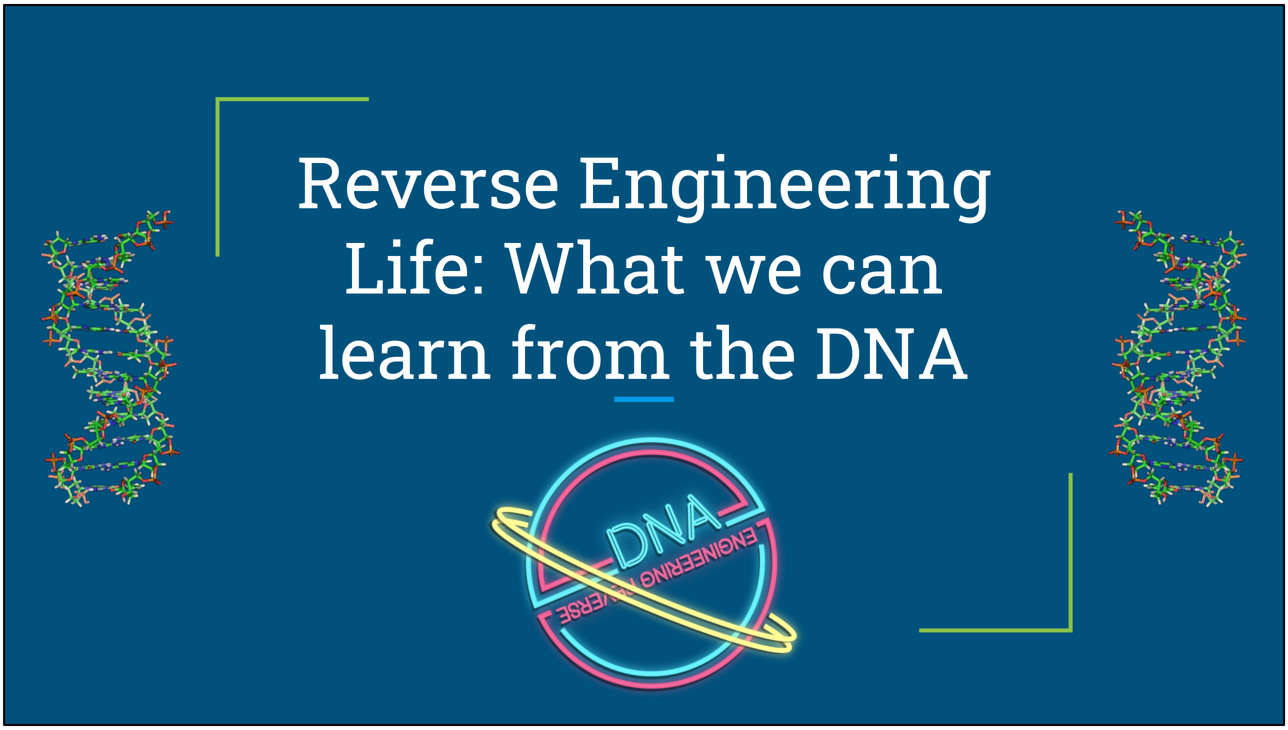


Reverse Engineering Life: What we can learn from the DNA

DNA
REVERSE ENGINEERING

Credits

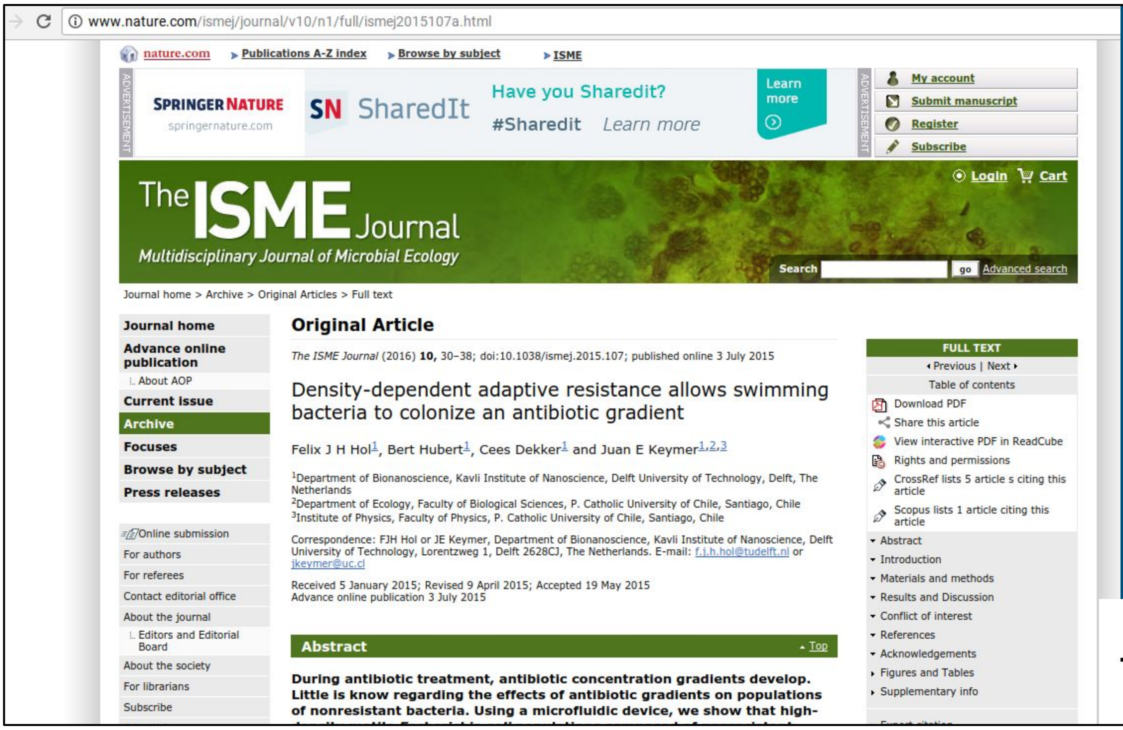
- This presentation leans heavily on other people's work and graphics
- All credits are available in the **speaker notes** which you should consult to find out who made all these great movies and images
- **Thank you so much Wikipedia Commons in particular!**

https://berthub.eu/revdna/

Online questions!

https://webchat.oftc.net/?channels=why2025-andromeda

IRC: oftc.net, channel: #why2025-andromeda



Original Article

The ISME Journal (2016) **10,** 30–38; doi:10.1038/ismej.2015.107; published online 3 July 2015

Density-dependent adaptive resistance allows swimming bacteria to colonize an antibiotic gradient

Felix J H Hol[1], Bert Hubert[1], Cees Dekker[1] and Juan E Keymer[1,2,3]

[1]Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, The Netherlands
[2]Department of Ecology, Faculty of Biological Sciences, P. Catholic University of Chile, Santiago, Chile
[3]Institute of Physics, Faculty of Physics, P. Catholic University of Chile, Santiago, Chile

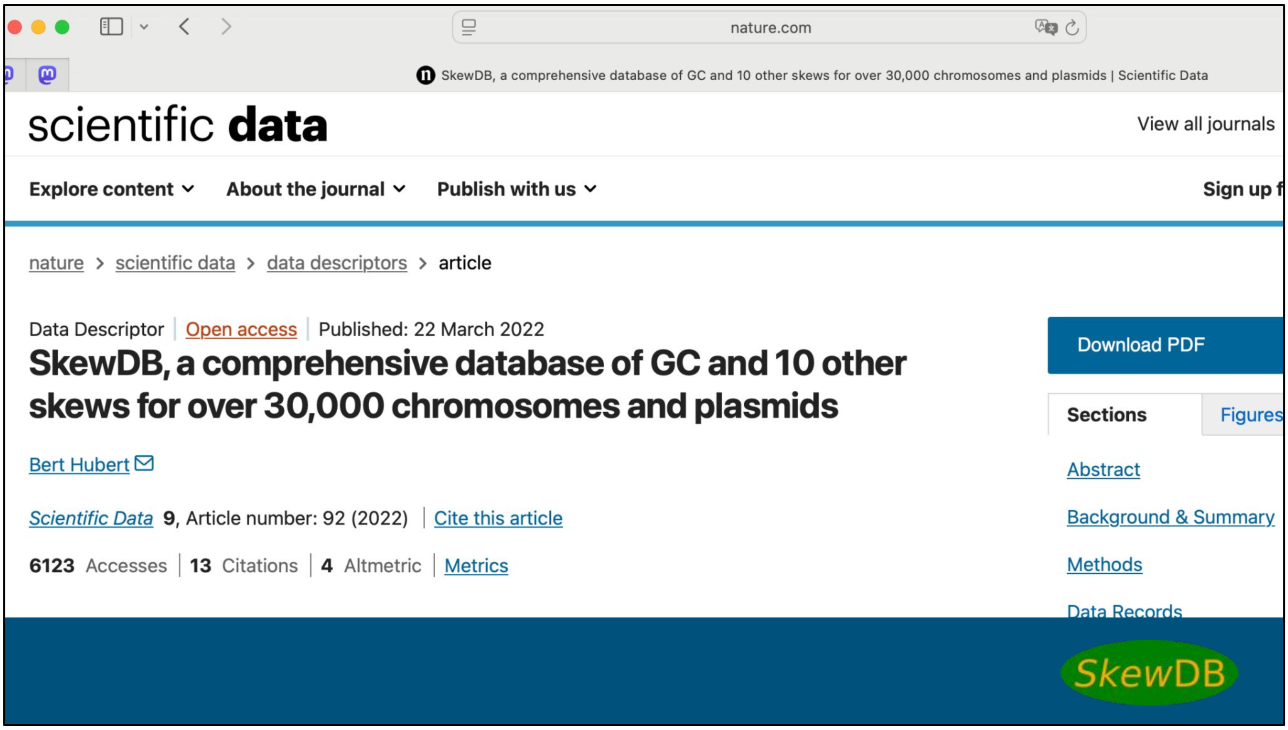
Correspondence: FJH Hol or JE Keymer, Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, Delft 2628CJ, The Netherlands. E-mail: f.j.h.hol@tudelft.nl or jkeymer@uc.cl

Received 5 January 2015; Revised 9 April 2015; Accepted 19 May 2015
Advance online publication 3 July 2015

Abstract

During antibiotic treatment, antibiotic concentration gradients develop. Little is know regarding the effects of antibiotic gradients on populations of nonresistant bacteria. Using a microfluidic device, we show that high-

https://www.nature.com/articles/ismej2015107



SkewDB, a comprehensive database of GC and 10 other skews for over 30,000 chromosomes and plasmids | Scientific Data

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nature > scientific data > data descriptors > article

Data Descriptor | Open access | Published: 22 March 2022

SkewDB, a comprehensive database of GC and 10 other skews for over 30,000 chromosomes and plasmids

Bert Hubert

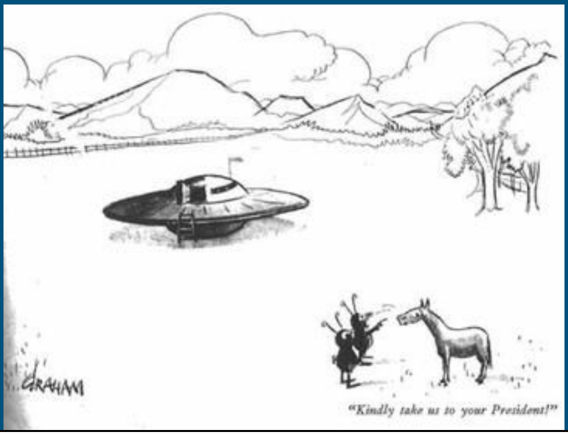
Scientific Data **9**, Article number: 92 (2022) | Cite this article

6123 Accesses | **13** Citations | **4** Altmetric | Metrics

https://www.nature.com/articles/s41597-022-01179-8

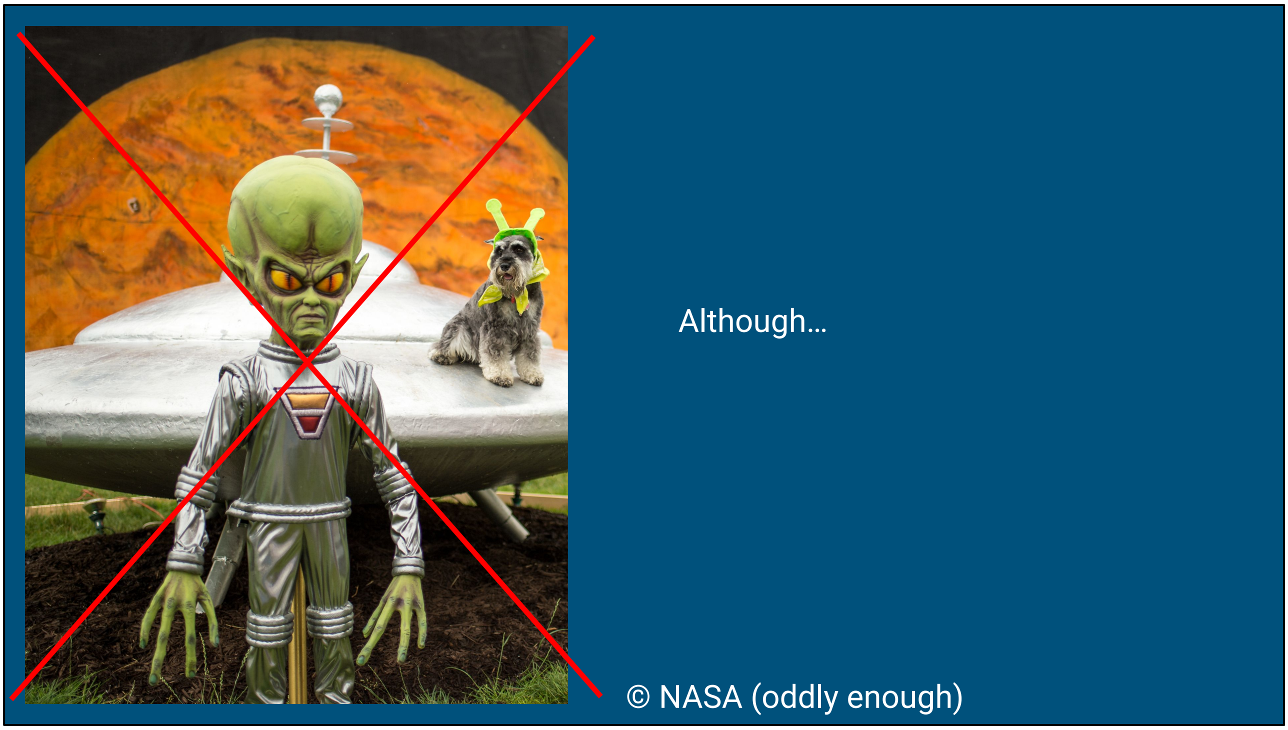
“Imagine a flashy spaceship lands in your backyard. The door opens and you are invited to investigate everything to see what you can learn. The technology is clearly millions of years beyond what we can make.

This is biology.”



“Kindly take us to your President!”

https://jsomers.net/i-should-have-loved-biology/

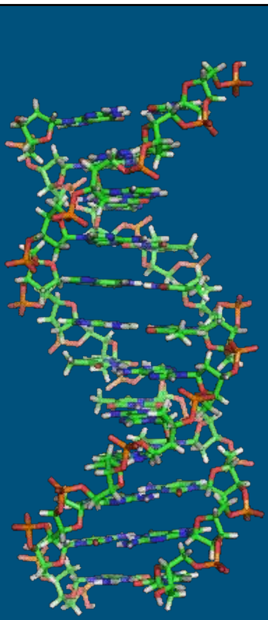


Although…

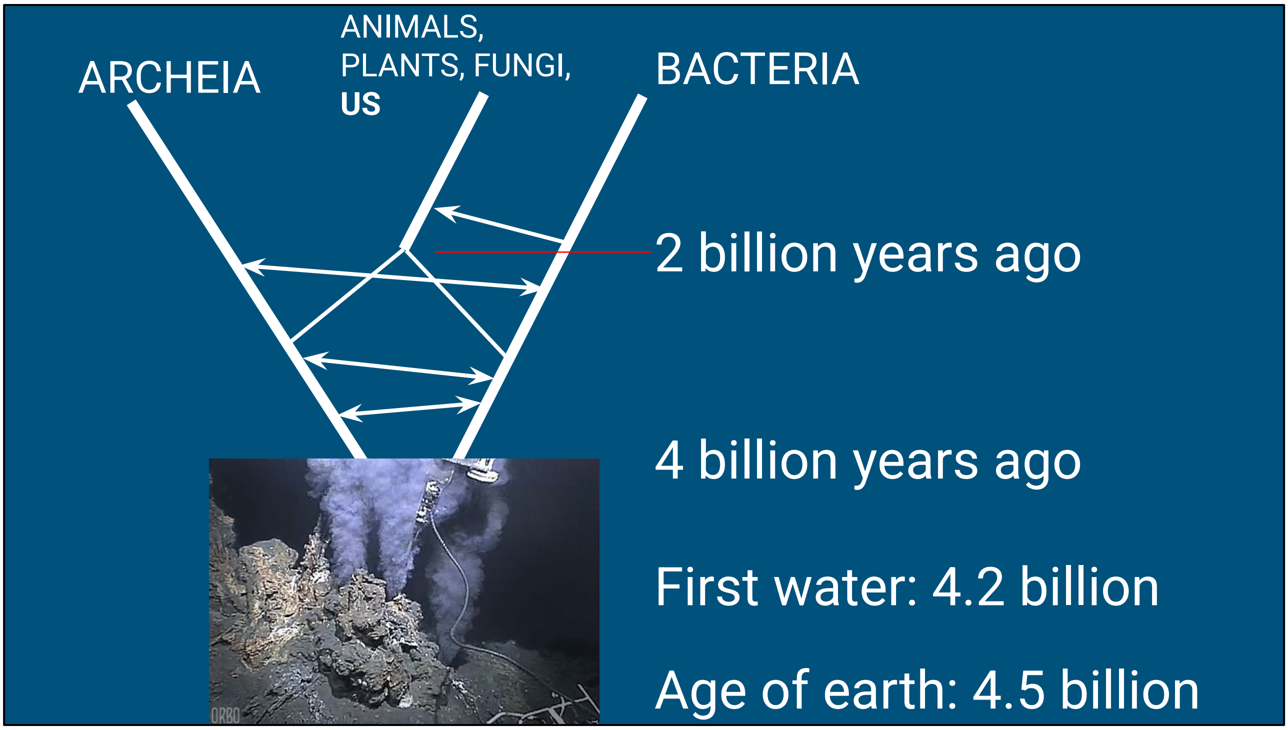
© NASA (oddly enough)

https://en.wikipedia.org/wiki/Little_green_men#/media/File:Mars_New_Year's_Celebration_(201506200007HQ)_(cropped).jpg

- Let’s study DNA the way we study random binary blobs
- Highlight many cool DNA things
- I want YOU to Join in!



- DNA: Millions, billions of nucleotides or “bases”:
    - A, C, G, T
- Organized in chromosomes & genes
- Absolutely **atom for atom** universal across all life
- >4 billion years old



ARCHEIA

ANIMALS, PLANTS, FUNGI, **US**

BACTERIA

2 billion years ago

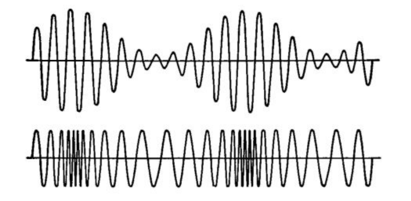
4 billion years ago

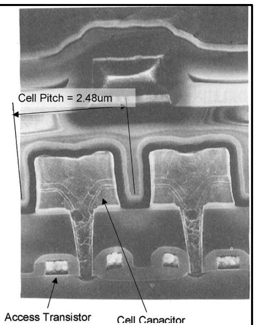
First water: 4.2 billion

Age of earth: 4.5 billion

https://giphy.com/gifs/sea-vents-hydrothermal-1bTEQnjArFBy8

Basics: DNA



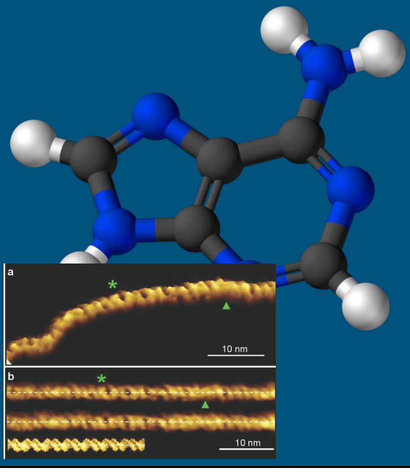


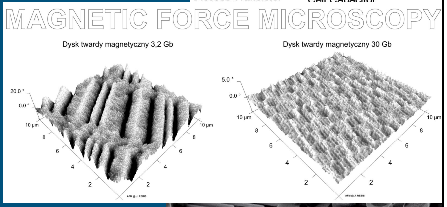
A “00”

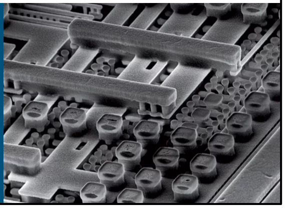
C “01”

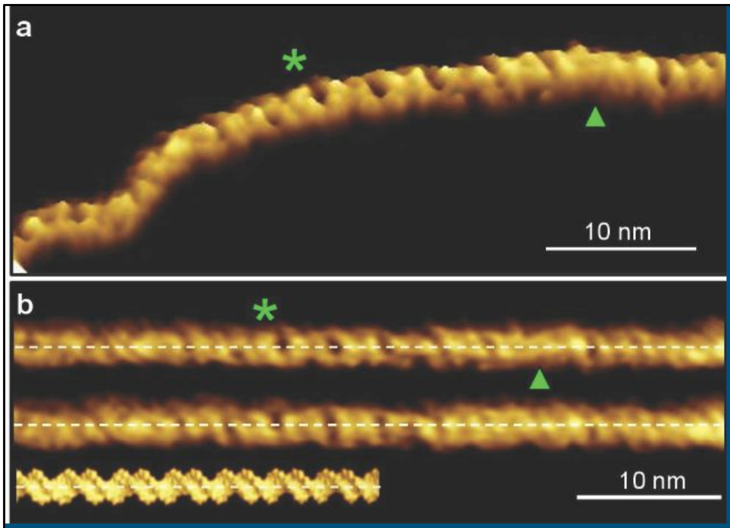
G “10”

T “11”









DNA is very much like tape

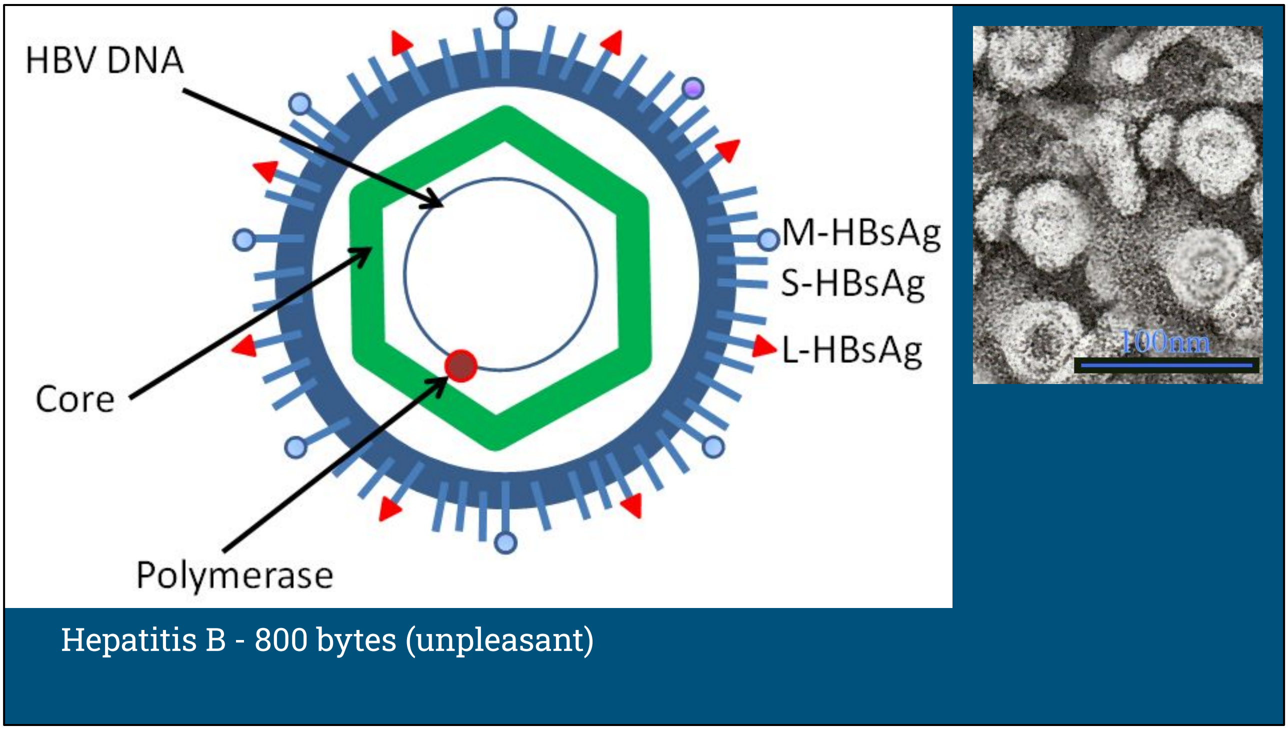
Sometimes circular tape - no beginning, no end!

No addressing! No alignment!

It is a **nucleotide stream** which can be compared to a **bitstream**

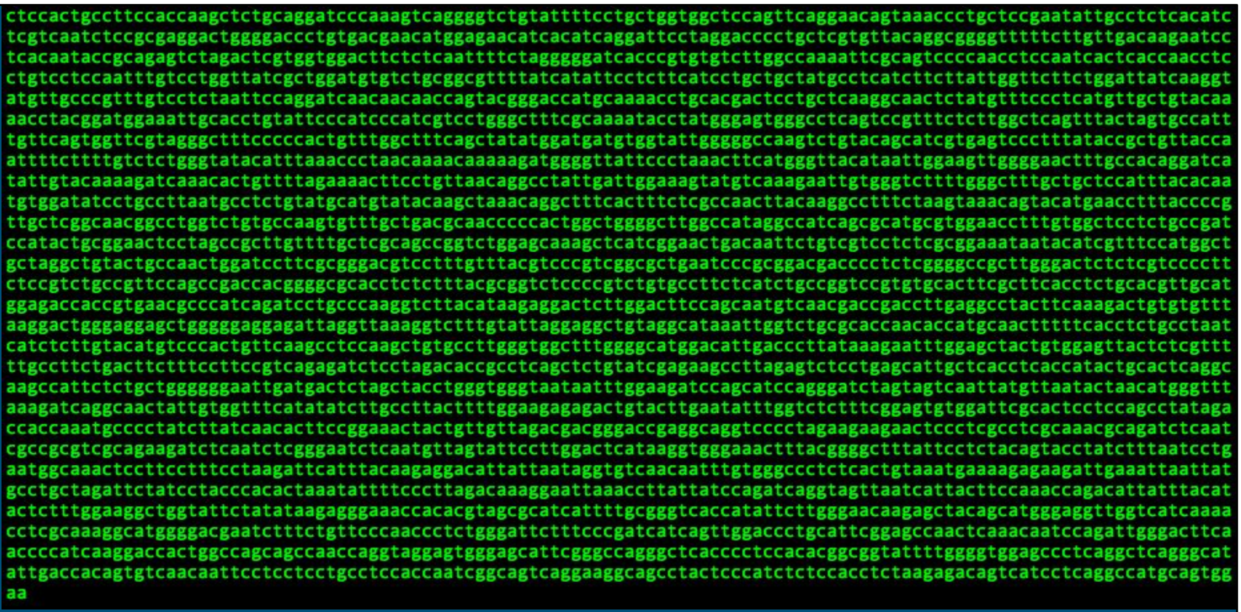
It IS however **content addressable**!

http://onlinelibrary.wiley.com/doi/10.1002/smll.201400265/full



Hepatitis B - 800 bytes (unpleasant)

By Dr Graham Beards - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=24121844
By GrahamColm at English Wikipedia, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=6032684



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aa
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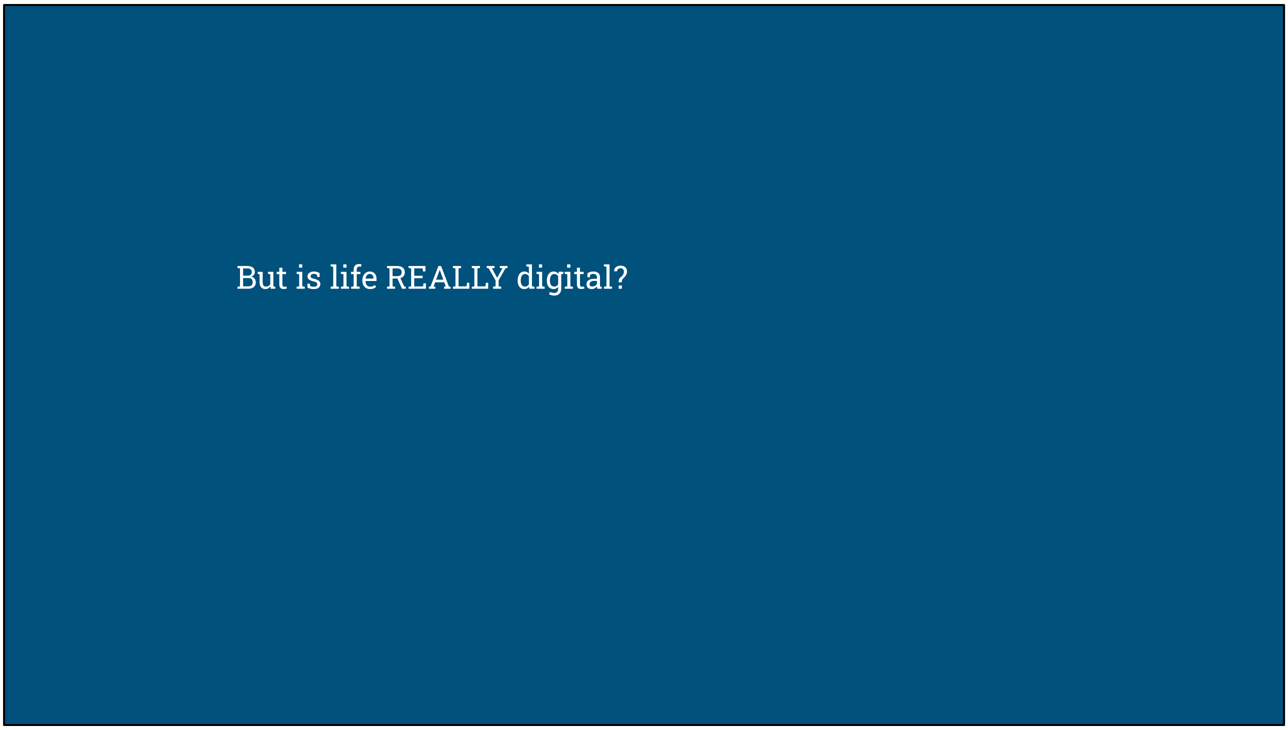
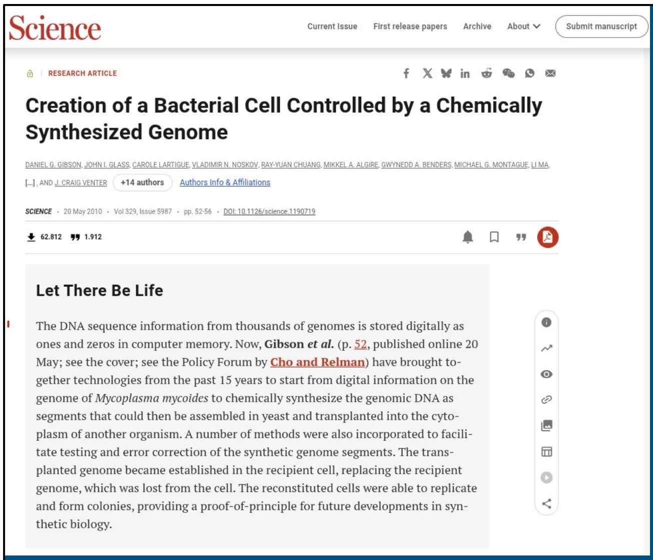
All of Hepatitis-B - 800 bytes



But is life REALLY digital?



RESEARCH ARTICLE

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

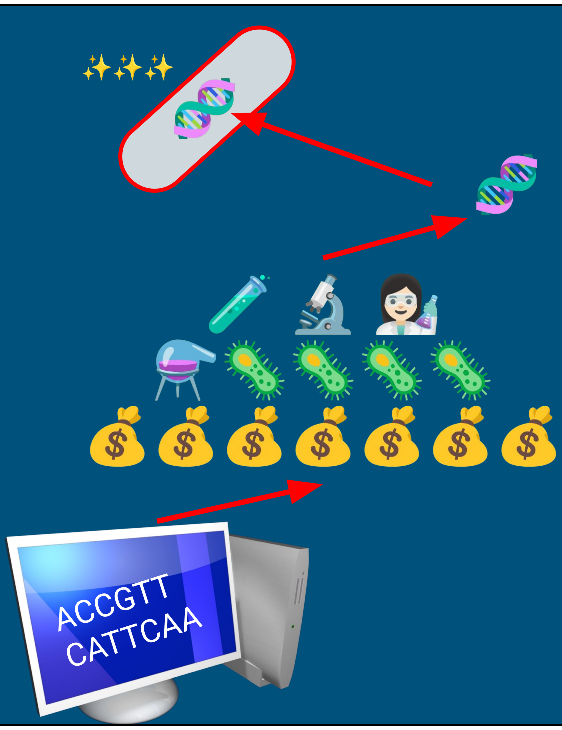
DANIEL G. GIBSON, JOHN I. GLASS, CAROLE LARTIGUE, VLADIMIR N. NOSKOV, RAY-YUAN CHUANG, MIKKEL A. ALGIRE, GWYNEDD A. BENDERS, MICHAEL G. MONTAGUE, LI MA, [...], AND J. CRAIG VENTER +14 authors Authors Info & Affiliations

SCIENCE • 20 May 2010 • Vol 329, Issue 5987 • pp. 52-56 • DOI: 10.1126/science.1190719

62.812 1.912

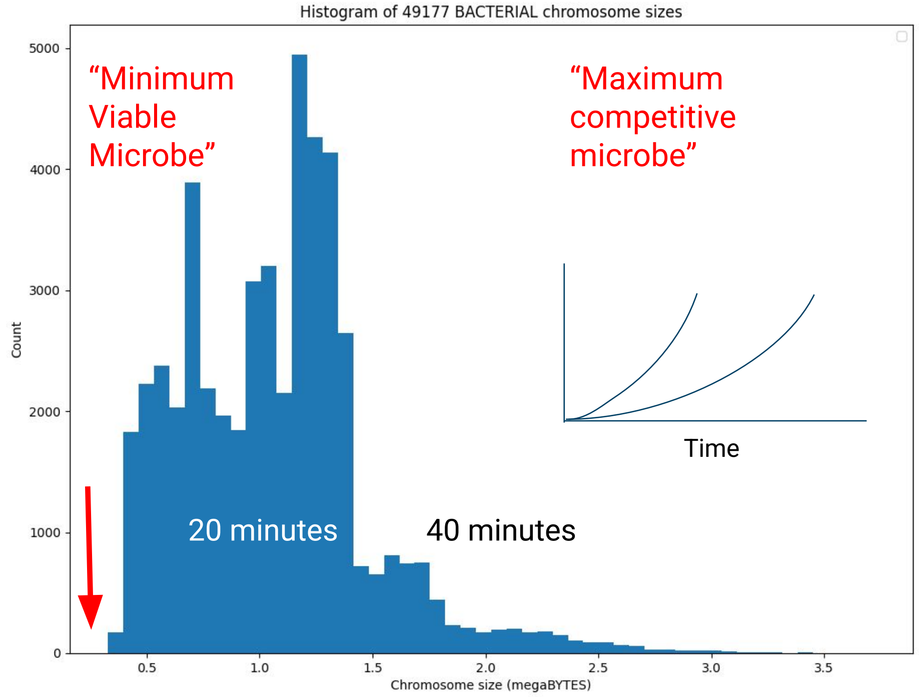
Let There Be Life

The DNA sequence information from thousands of genomes is stored digitally as ones and zeros in computer memory. Now, **Gibson *et al.*** (p. 52, published online 20 May; see the cover; see the Policy Forum by **Cho and Relman**) have brought together technologies from the past 15 years to start from digital information on the genome of *Mycoplasma mycoides* to chemically synthesize the genomic DNA as segments that could then be assembled in yeast and transplanted into the cytoplasm of another organism. A number of methods were also incorporated to facilitate testing and error correction of the synthetic genome segments. The transplanted genome became established in the recipient cell, replacing the recipient genome, which was lost from the cell. The reconstituted cells were able to replicate and form colonies, providing a proof-of-principle for future developments in synthetic biology.



But is life DIGITAL?

https://www.science.org/doi/10.1126/science.1190719

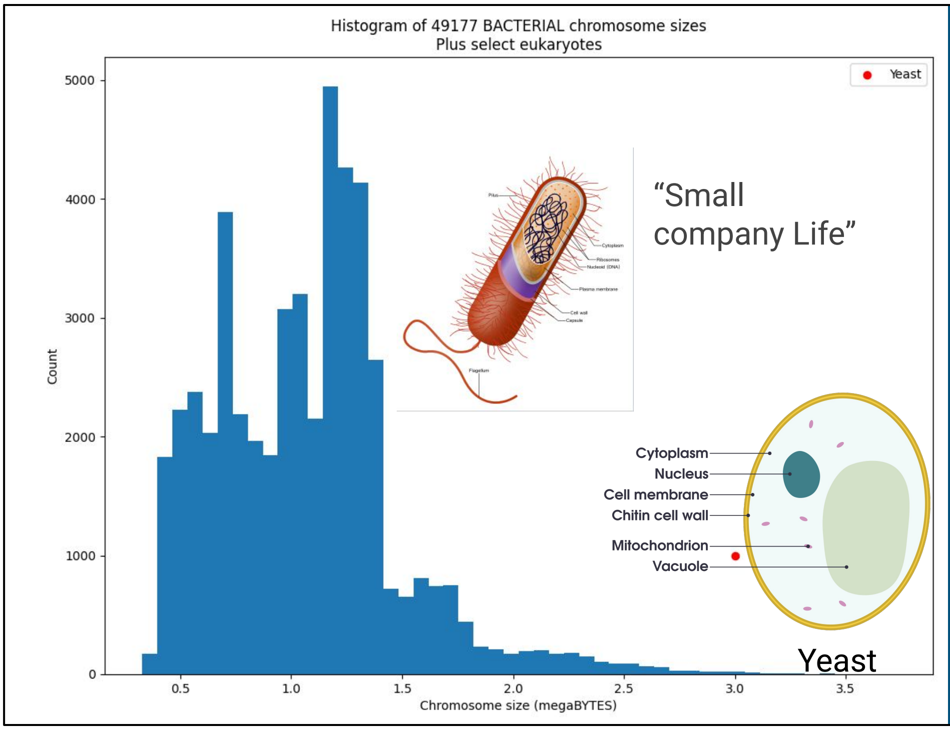


“Minimum Viable Microbe”

“Maximum competitive microbe”

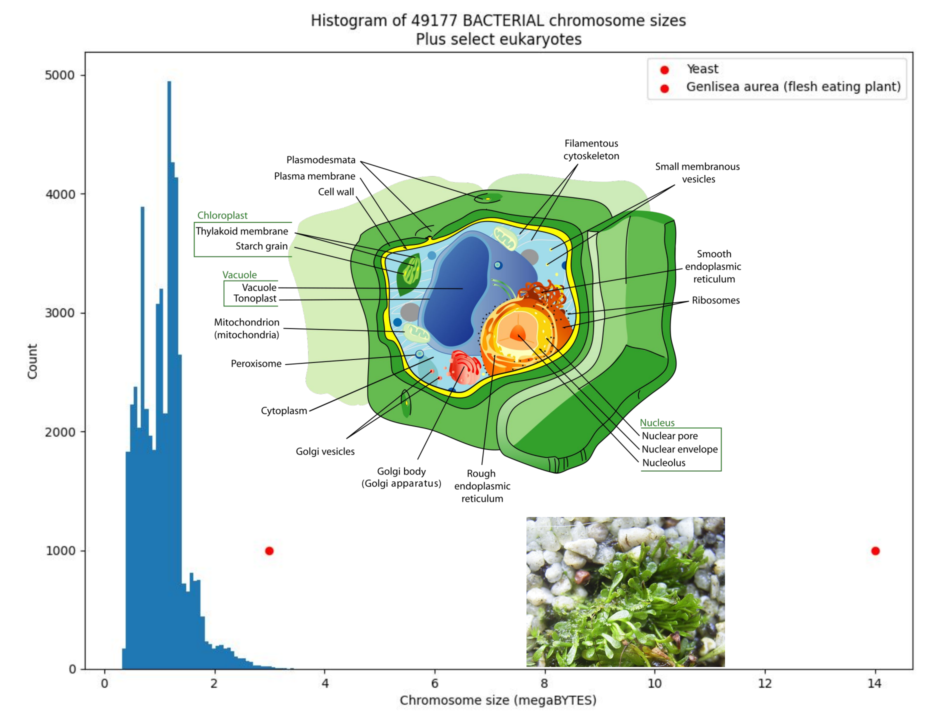
20 minutes

40 minutes



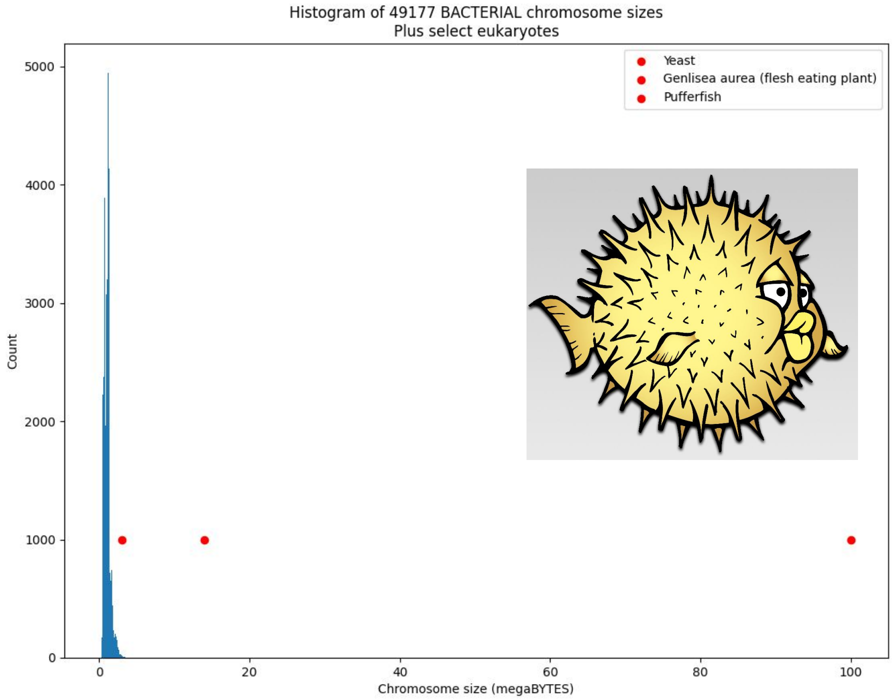
“Small company Life”

“Enterprise Life” - various departments and procedures

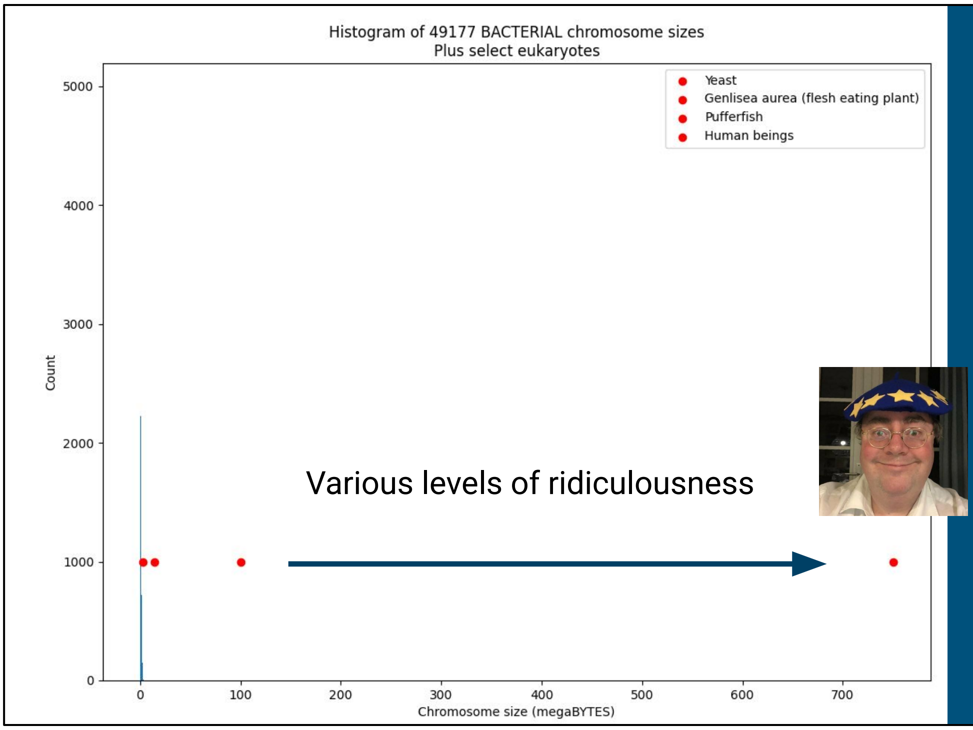


By LadyofHats - Self-made using Adobe Illustrator. https://commons.wikimedia.org/w/index.php?curid=844682

“Mega-corp territory”

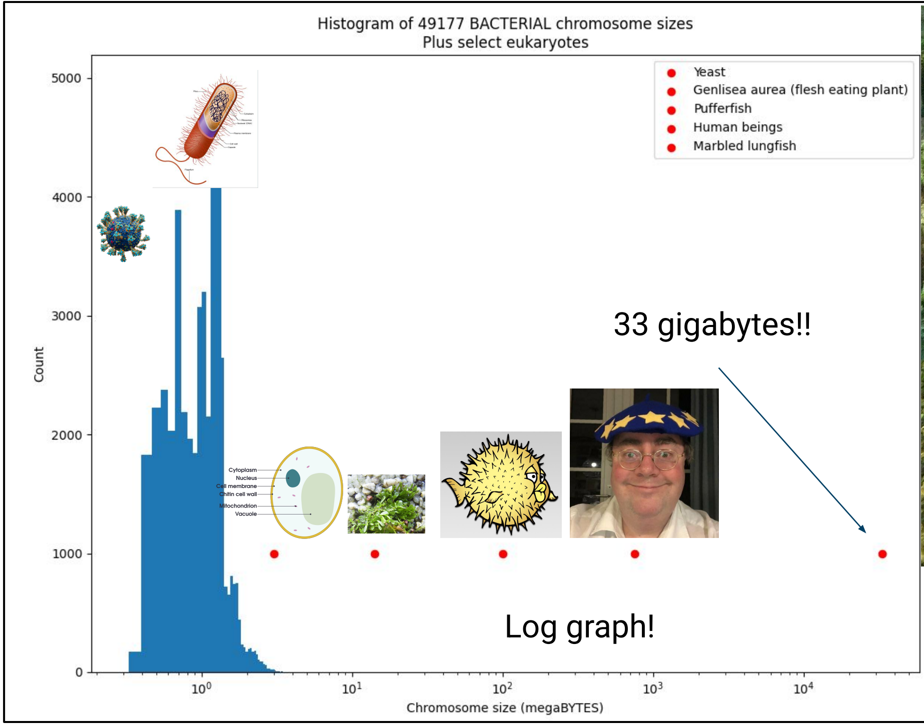


Most compact animal. Genome almost completely used for proteins.



Various levels of ridiculousness

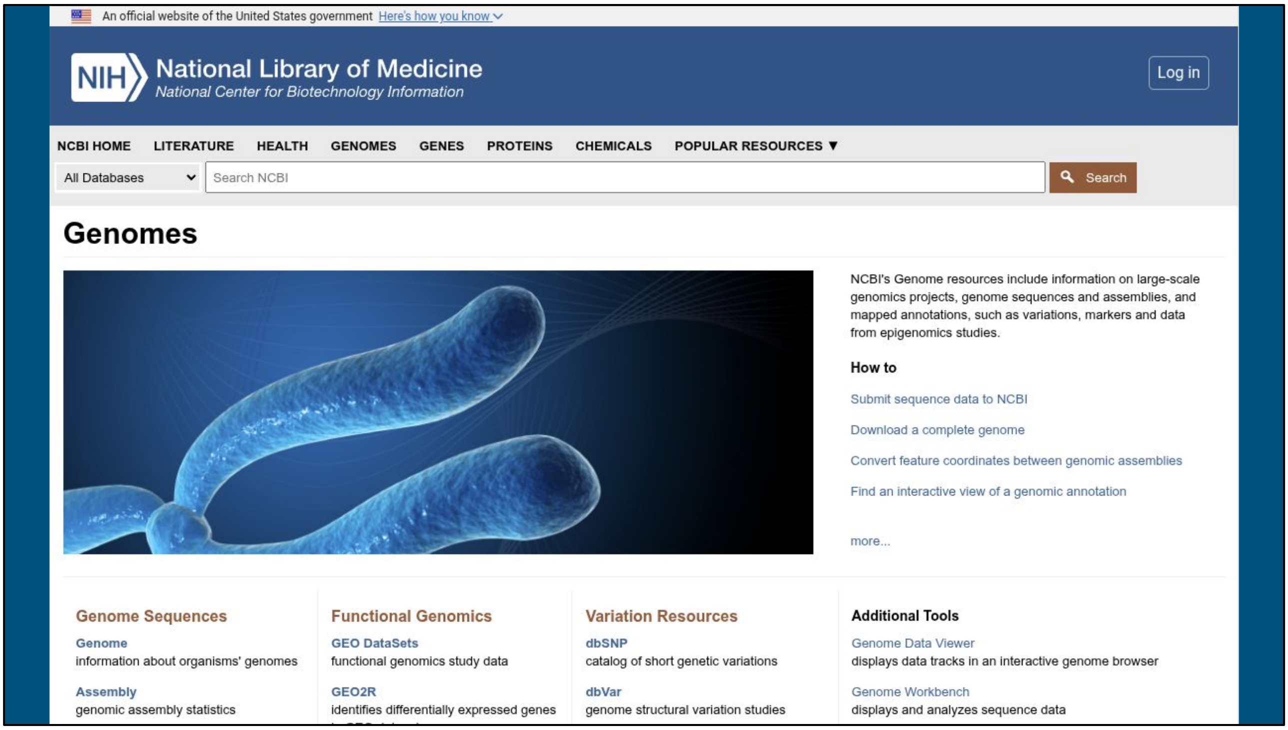
“Bloated mega-corp DNA”



33 gigabytes!!

Log graph!

Unimaginably over the top bloated megacorp stuff!



An official website of the United States government Here's how you know

NIH National Library of Medicine

National Center for Biotechnology Information

Genomes

NCBI's Genome resources include information on large-scale genomics projects, genome sequences and assemblies, and mapped annotations, such as variations, markers and data from epigenomics studies.

How to

- Submit sequence data to NCBI
- Download a complete genome
- Convert feature coordinates between genomic assemblies
- Find an interactive view of a genomic annotation

more...

Genome Sequences

Genome
information about organisms' genomes

Assembly
genomic assembly statistics

Functional Genomics

GEO DataSets
functional genomics study data

GEO2R
identifies differentially expressed genes

Variation Resources

dbSNP
catalog of short genetic variations

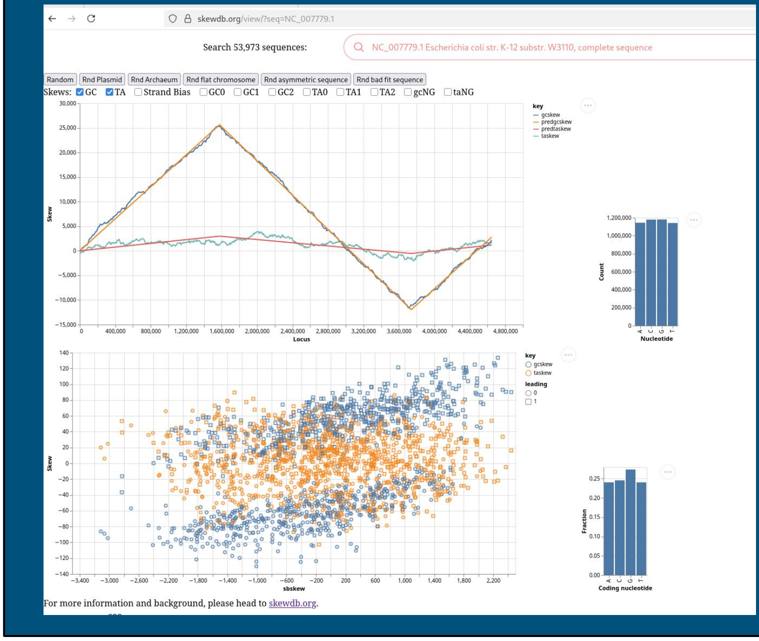
dbVar
genome structural variation studies

Additional Tools

Genome Data Viewer
displays data tracks in an interactive genome browser

Genome Workbench
displays and analyzes sequence data

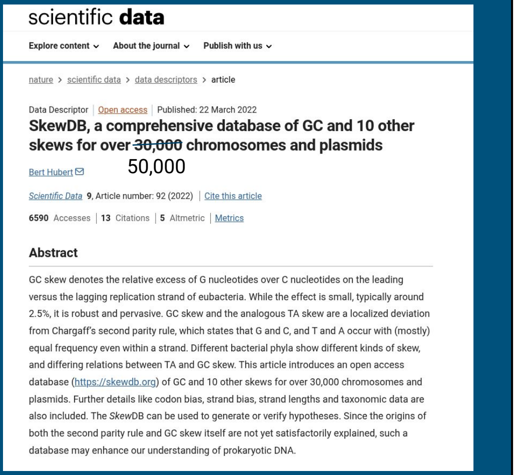
https://skewdb.org/ - https://skewdb.org/view/ - CSV FILES!



Search 53,973 sequences: NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete sequence

Skews: ☑ GC ☑ TA ☐ Strand Bias ☐ GC0 ☐ GC1 ☐ GC2 ☐ TA0 ☐ TA1 ☐ TA2 ☐ gcNG ☐ taNG

For more information and background, please head to skewdb.org.



nature > scientific data > data descriptors > article

Data Descriptor | Open access | Published: 22 March 2022

SkewDB, a comprehensive database of GC and 10 other skews for over ~~30,000~~ chromosomes and plasmids

50,000

Bert Hubert

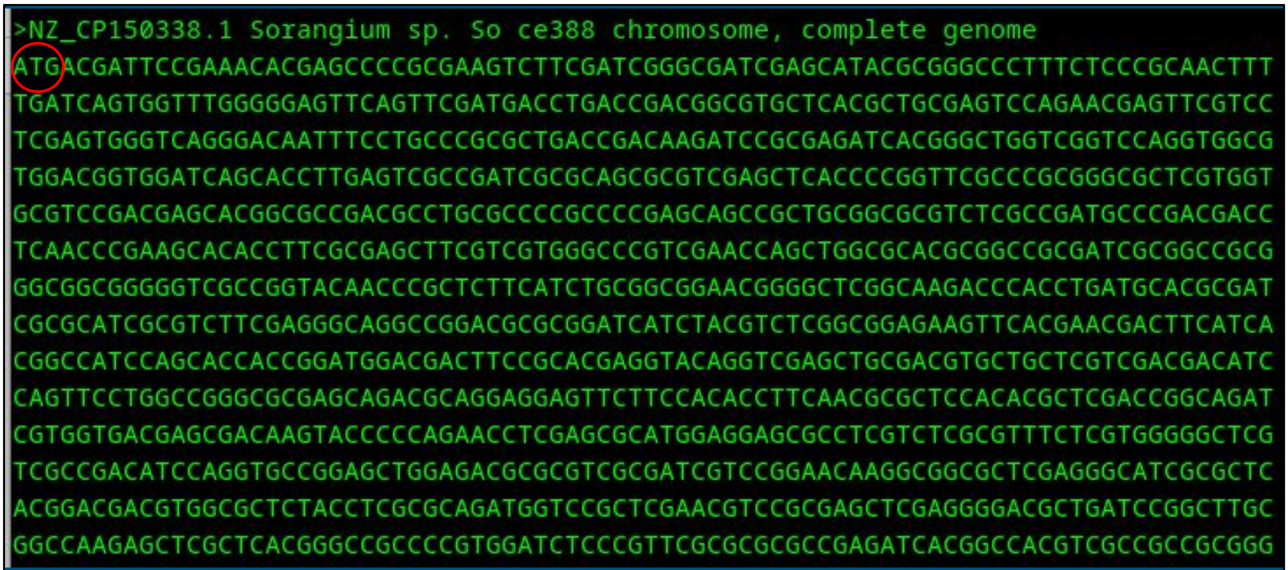
Scientific Data **9**, Article number: 92 (2022) | Cite this article

6590 Accesses | **13** Citations | **5** Altmetric | Metrics

Abstract

GC skew denotes the relative excess of G nucleotides over C nucleotides on the leading versus the lagging replication strand of eubacteria. While the effect is small, typically around 2.5%, it is robust and pervasive. GC skew and the analogous TA skew are a localized deviation from Chargaff's second parity rule, which states that G and C, and T and A occur with (mostly) equal frequency even within a strand. Different bacterial phyla show different kinds of skew, and differing relations between TA and GC skew. This article introduces an open access database (https://skewdb.org) of GC and 10 other skews for over 30,000 chromosomes and plasmids. Further details like codon bias, strand bias, strand lengths and taxonomic data are also included. The *SkewDB* can be used to generate or verify hypotheses. Since the origins of both the second parity rule and GC skew itself are not yet satisfactorily explained, such a database may enhance our understanding of prokaryotic DNA.

Let’s dive into the >50,000 binaries!
(in ASCII)



```
>NZ_CP150338.1 Sorangium sp. So ce388 chromosome, complete genome
ATGACGATTCCGAAACACGAGCCCCGCGAAGTCTTCGATCGGGCGATCGAGCATACGCGGGCCCTTTCTCCCGCAACTTT
TGATCAGTGGTTTGGGGGAGTTCAGTTCGATGACCTGACCGACGGCGTGCTCACGCTGCGAGTCCAGAACGAGTTCGTCC
TCGAGTGGGTCAGGGACAATTTCCTGCCCGCGCTGACCGACAAGATCCGCGAGATCACGGGCTGGTCGGTCCAGGTGGCG
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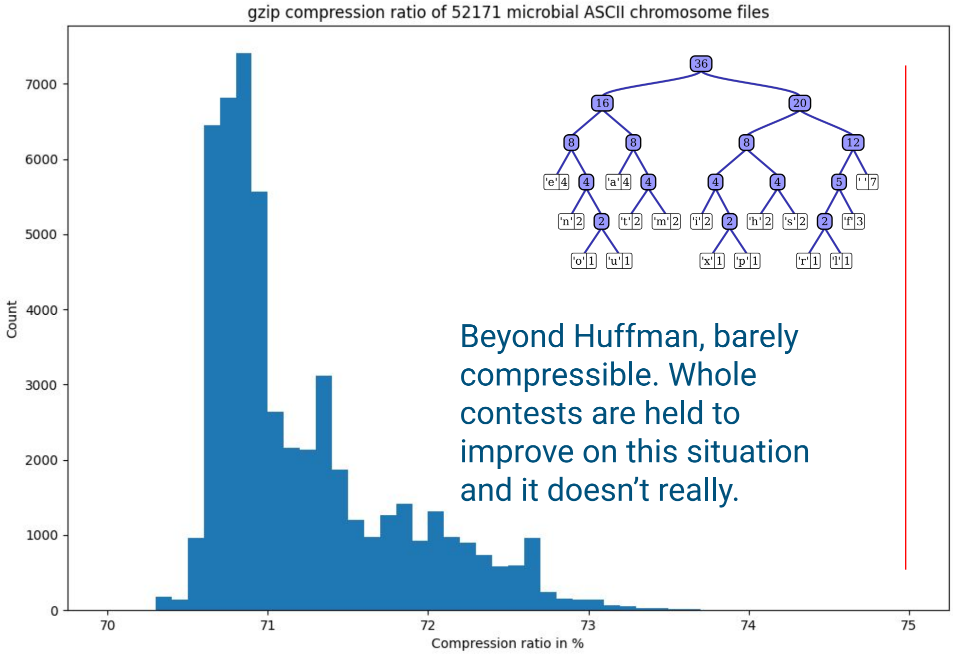
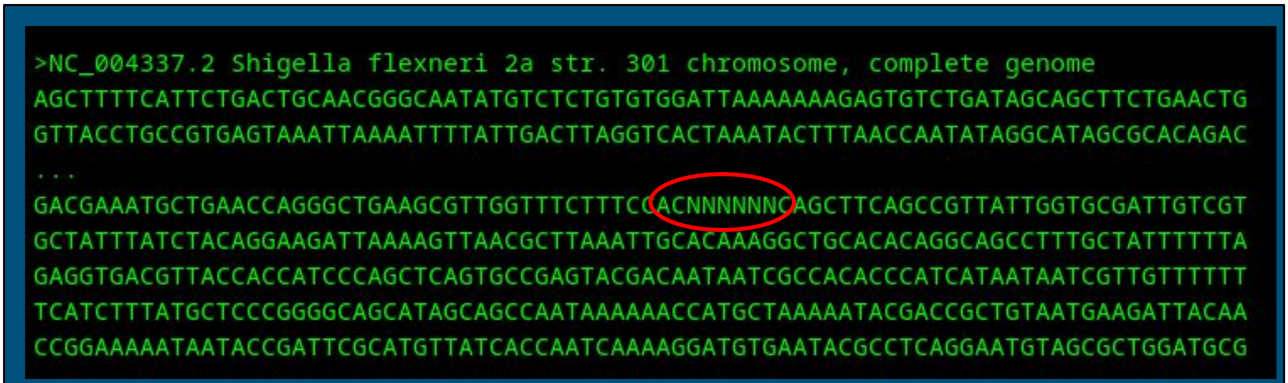
https://ftp.ncbi.nlm.nih.gov/genomes/refseq/
GCF_051474125.1_ASM5147412v1_genomic.fna.**gz**



Beyond Huffman, barely compressible. Whole contests are held to improve on this situation and it doesn’t really.



```
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...
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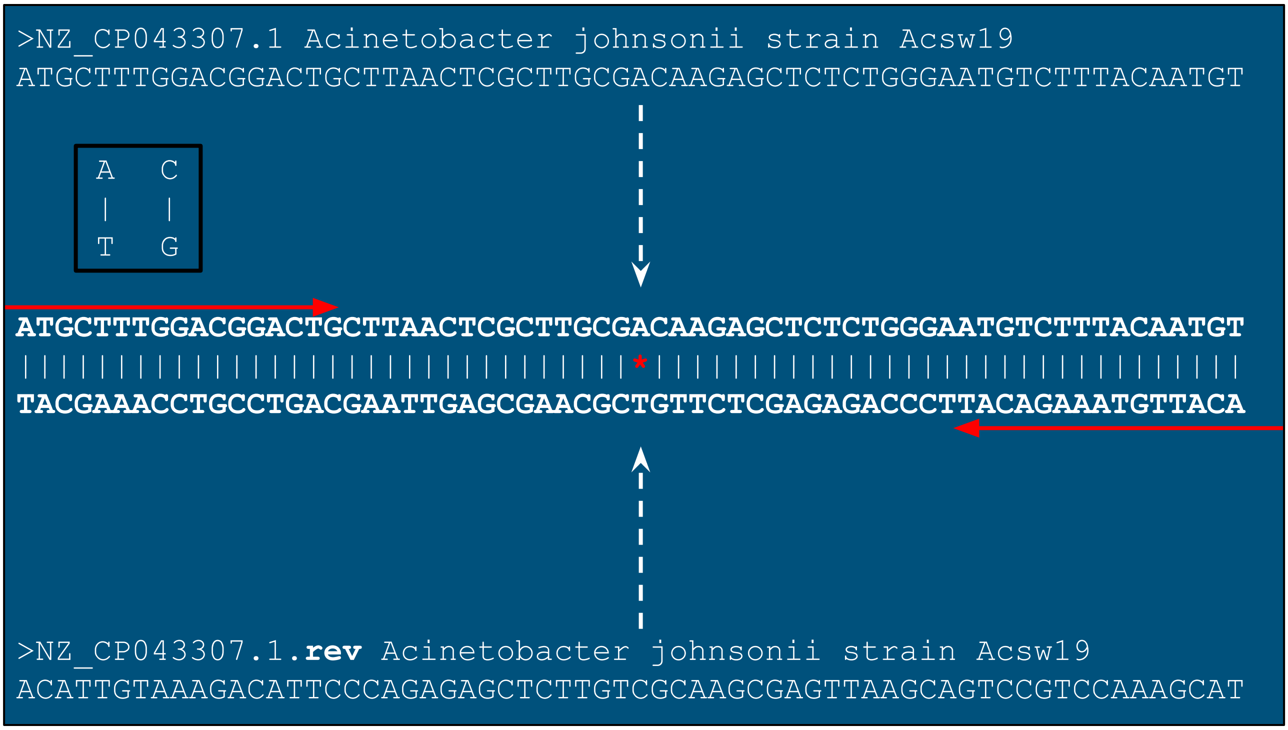
GAGGTGACGTTACCACCATCCCAGCTCAGTGCCGAGTACGACAATAATCGCCACACCCATCATAATAATCGTTGTTTTTT
TCATCTTTATGCTCCCGGGGCAGCATAGCAGCCAATAAAAAACCATGCTAAAAATACGACCGCTGTAATGAAGATTACAA
CCGGAAAAATAATACCGATTCGCATGTTATCACCAATCAAAAGGATGTGAATACGCCTCAGGAATGTAGCGCTGGATGCG
```

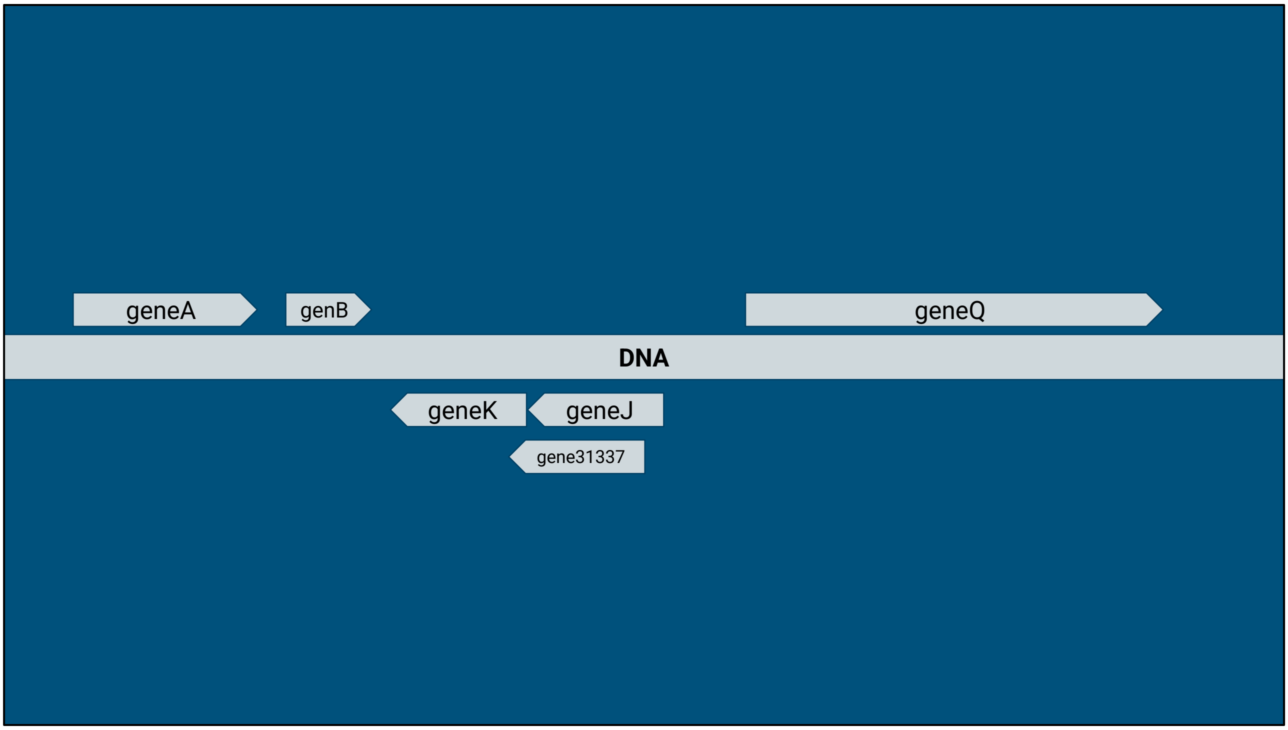
“2bit” formats would achieve 75% compression, and allow better further compression, but the field doesn’t care.

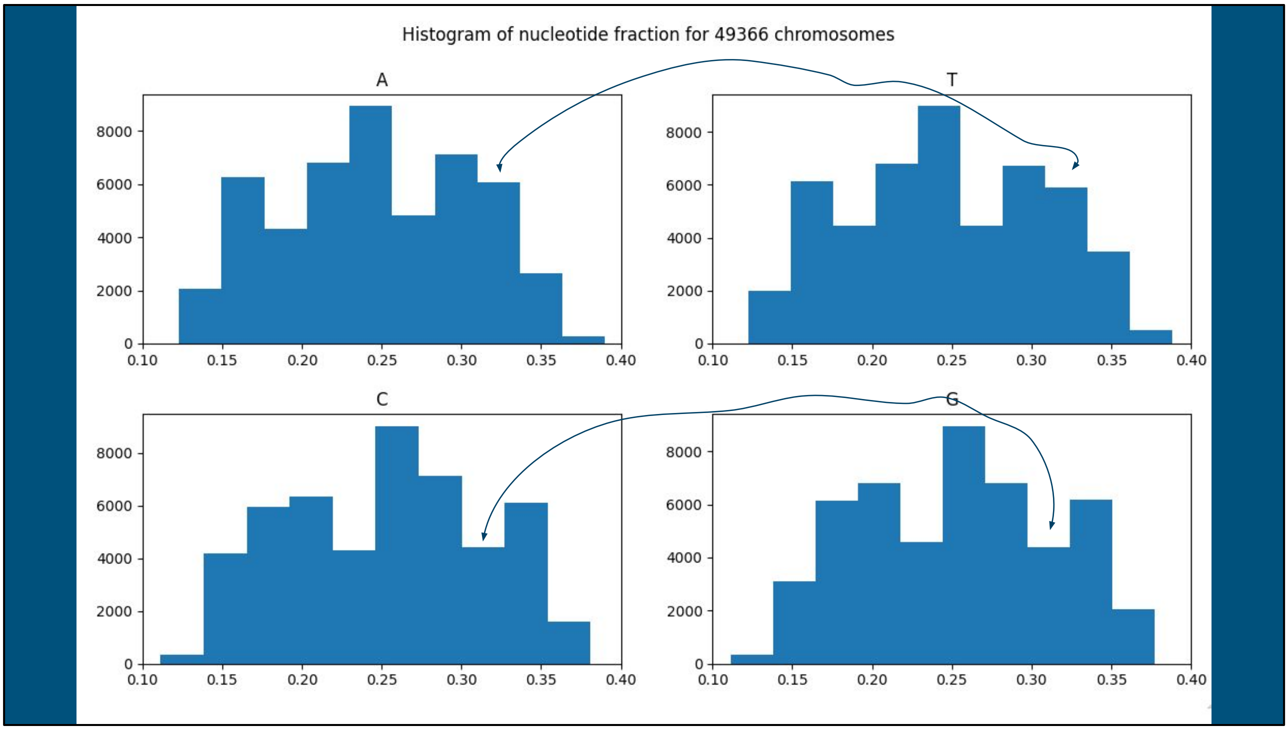


>NZ_CP043307.1 Acinetobacter johnsonii strain Acsw19
ATGCTTTGGACGGACTGCTTAACTCGCTTGCGACAAGAGCTCTCTGGGAATGTCTTTACAATGT

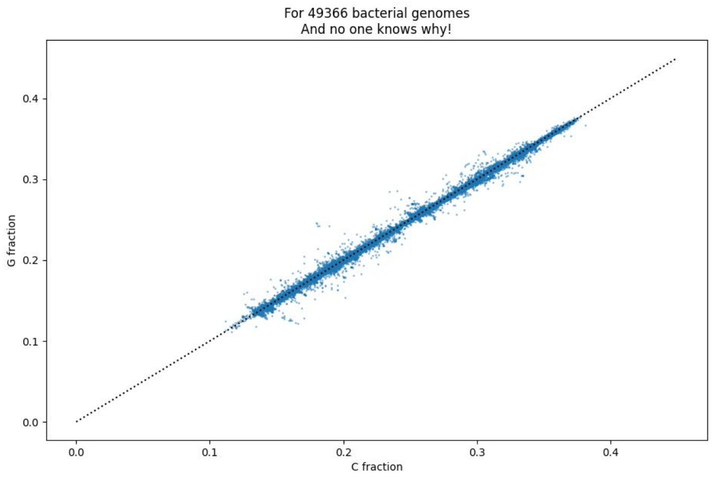
```
ATGCTTTGGACGGACTGCTTAACTCGCTTGCGACAAGAGCTCTCTGGGAATGTCTTTACAATGT
||||||||||||||||||||||||||||||||*|||||||||||||||||||||||||||||||
TACGAAACCTGCCTGACGAATTGAGCGAACGCTGTTCTCGAGAGACCCTTACAGAAATGTTACA
```

>NZ_CP043307.1.**rev** Acinetobacter johnsonii strain Acsw19
ACATTGTAAAGACATTCCCAGAGAGCTCTTGTCGCAAGCGAGTTAAGCAGTCCGTCCAAAGCAT

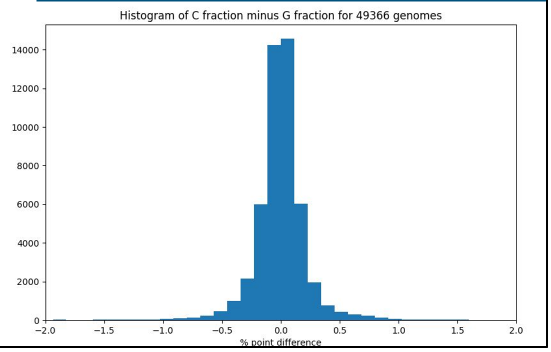

geneA
genB
geneQ
DNA
geneK
geneJ
gene31337



Histogram of nucleotide fraction for 49366 chromosomes



“The second Chargaff rule holds that both A% ≈ T% and G% ≈ C% are valid for **each** of the two DNA strands”



“The basis for this rule is still under investigation” (!!!)

https://en.wikipedia.org/wiki/Chargaff%27s_rules

But wait, it gets weirder

Complementary DNA:

C -> G
A -> T

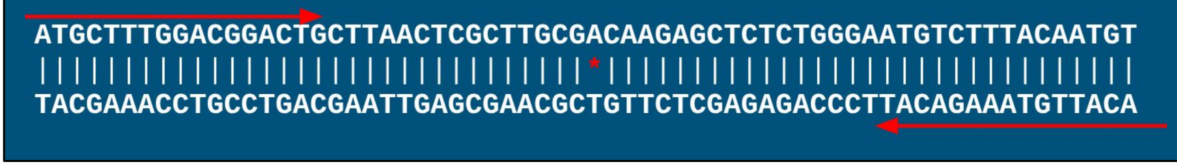
Reverse & complement:

CTA -> GAT -> **TAG**

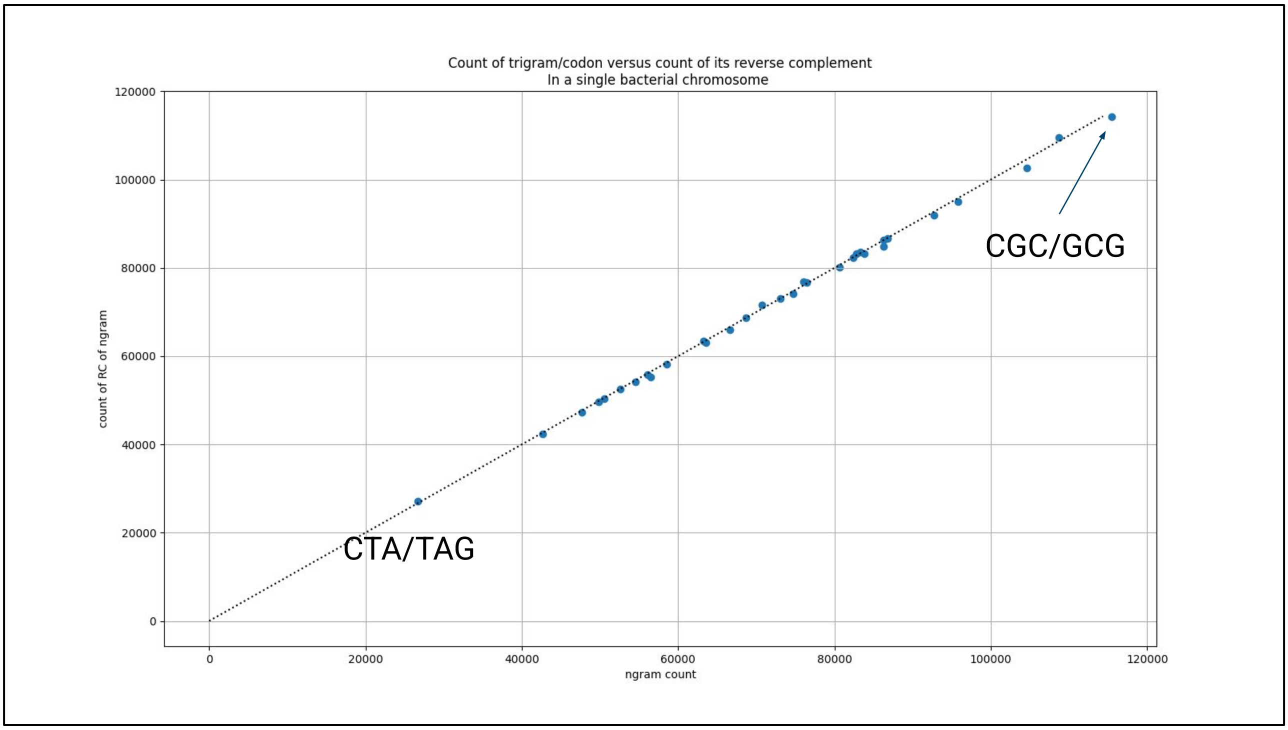
ATG -> TAC -> CAT

- Do the complement thing
- Reverse the string

“Reverse Complement” (RC)

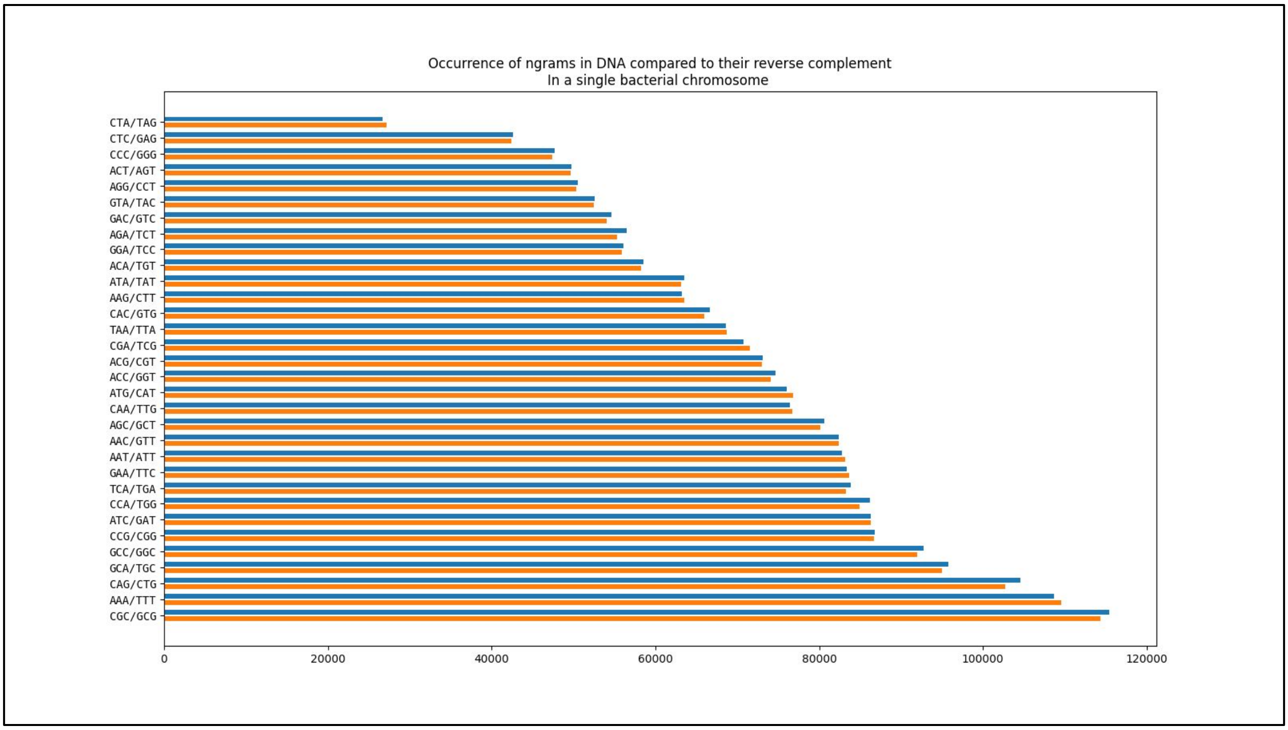


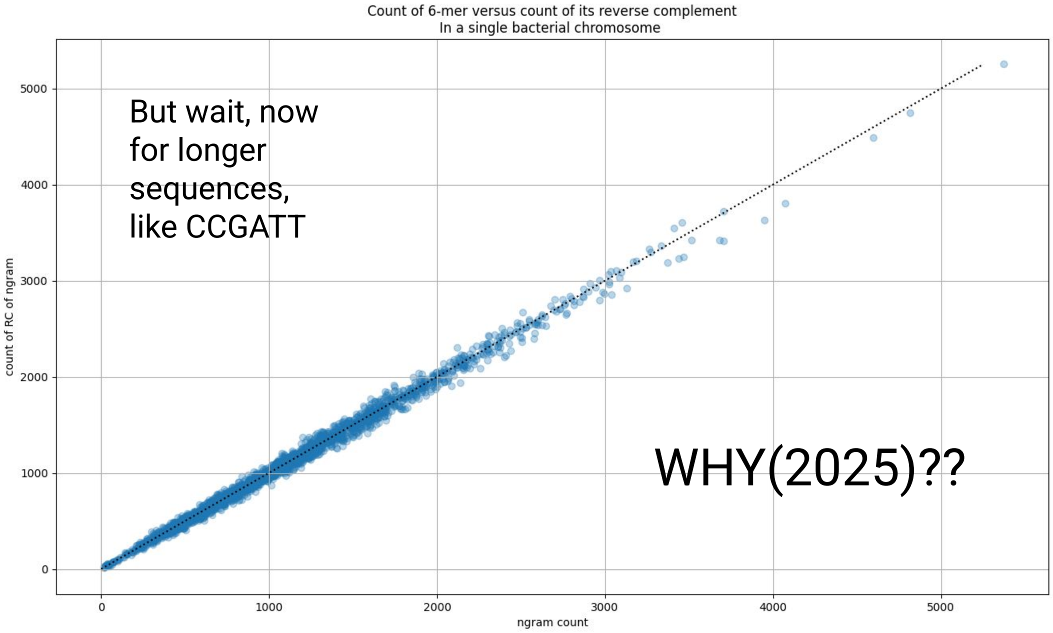
```
ATGCTTTGGACGGACTGCTTAACTCGCTTGCGACAAGAGCTCTCTGGGAATGTCTTTACAATGT
||||||||||||||||||||||||||||||||*|||||||||||||||||||||||||||||||
TACGAAACCTGCCTGACGAATTGAGCGAACGCTGTTCTCGAGAGACCCTTACAGAAATGTTACA
```



CGC/GCG

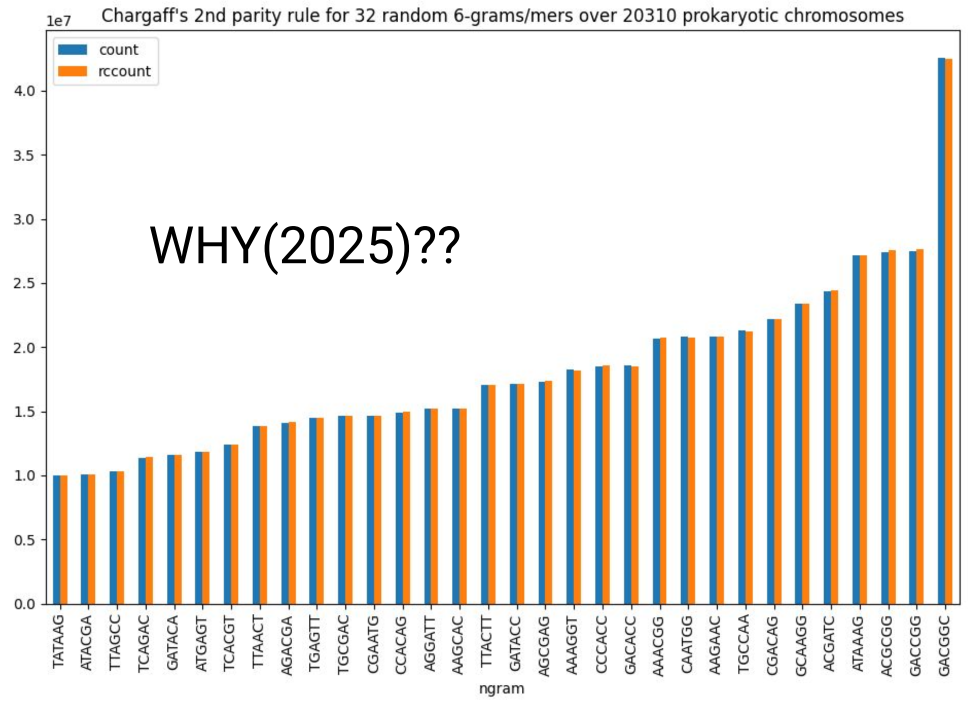
CTA/TAG


Occurrence of ngrams in DNA compared to their reverse complement
In a single bacterial chromosome
CTA/TAG
CTC/GAG
CCC/GGG
ACT/AGT
AGG/CCT
GTA/TAC
GAC/GTC
AGA/TCT
GGA/TCC
ACA/TGT
ATA/TAT
AAG/CTT
CAC/GTG
TAA/TTA
CGA/TCG
ACG/CGT
ACC/GGT
ATG/CAT
CAA/TTG
AGC/GCT
AAC/GTT
AAT/ATT
GAA/TTC
TCA/TGA
CCA/TGG
ATC/GAT
CCG/CGG
GCC/GGC
GCA/TGC
CAG/CTG
AAA/TTT
CGC/GCG
0
20000
40000
60000
80000
100000
120000



But wait, now for longer sequences, like CCGATT

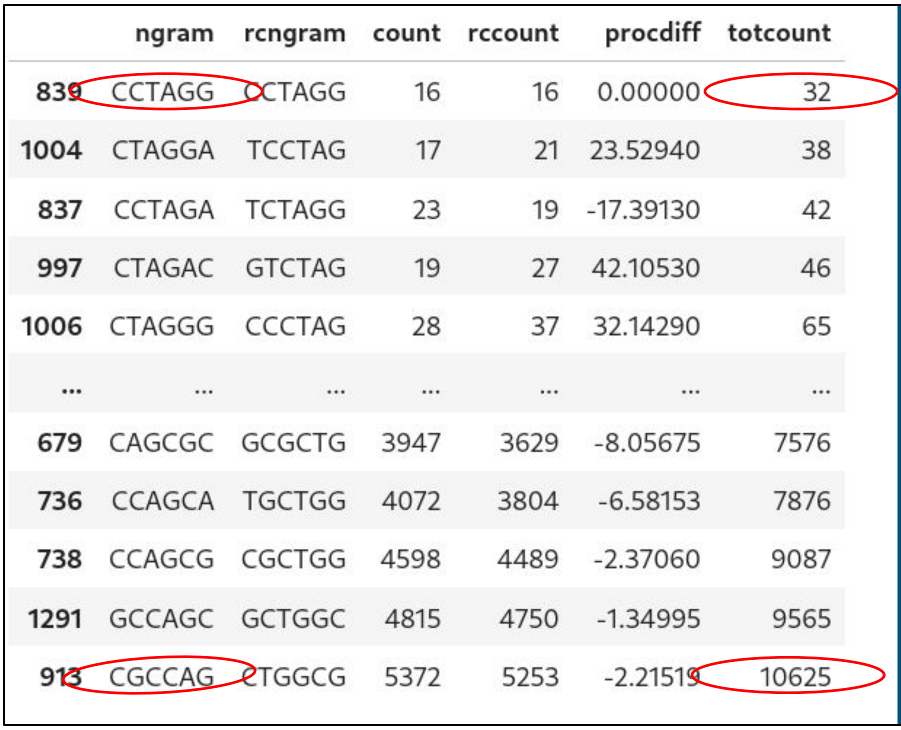
WHY(2025)??



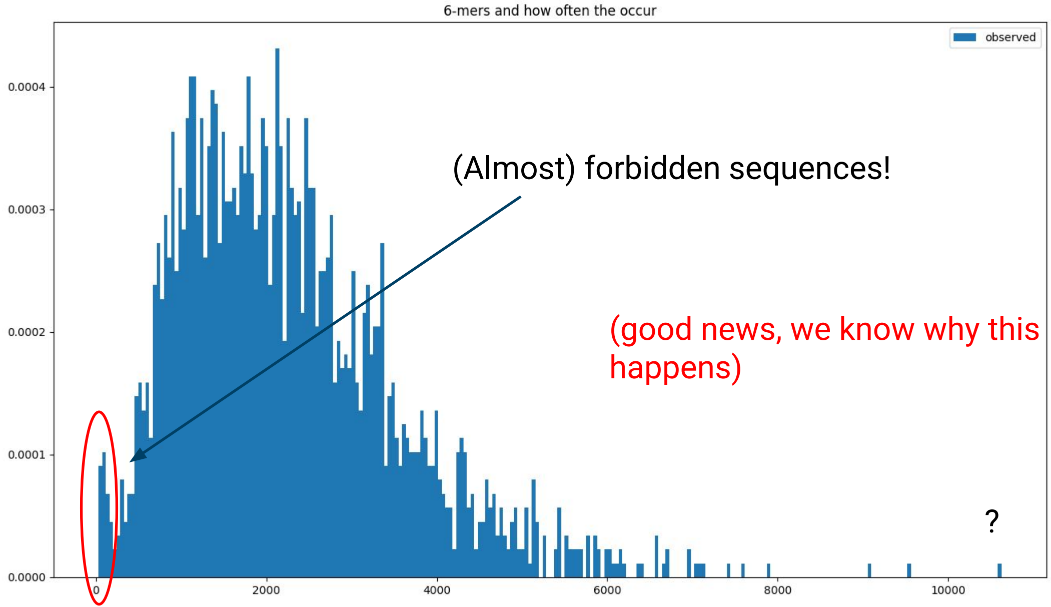
WHY(2025)??

We need to definitively find out why this is so, and if it means something!

It works up to *10* characters at least.



	ngram	rcngram	count	rccount	procdiff	totcount
839	CCTAGG	CCTAGG	16	16	0.00000	32
1004	CTAGGA	TCCTAG	17	21	23.52940	38
837	CCTAGA	TCTAGG	23	19	-17.39130	42
997	CTAGAC	GTCTAG	19	27	42.10530	46
1006	CTAGGG	CCCTAG	28	37	32.14290	65
...	...	...	...	...	...	...
679	CAGCGC	GCGCTG	3947	3629	-8.05675	7576
736	CCAGCA	TGCTGG	4072	3804	-6.58153	7876
738	CCAGCG	CGCTGG	4598	4489	-2.37060	9087
1291	GCCAGC	GCTGGC	4815	4750	-1.34995	9565
913	CGCCAG	CTGGCG	5372	5253	-2.21519	10625



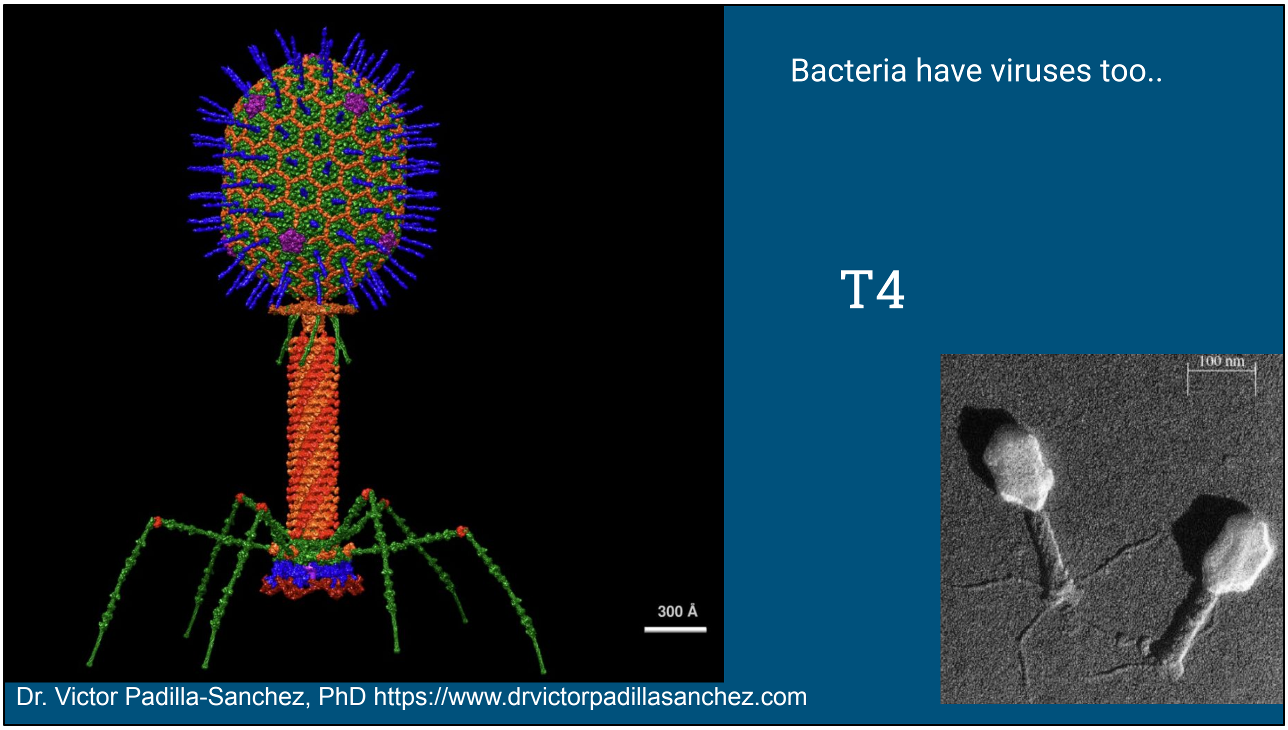
(Almost) forbidden sequences!

(good news, we know why this happens)

?

CCTAGG…

CGCCAG

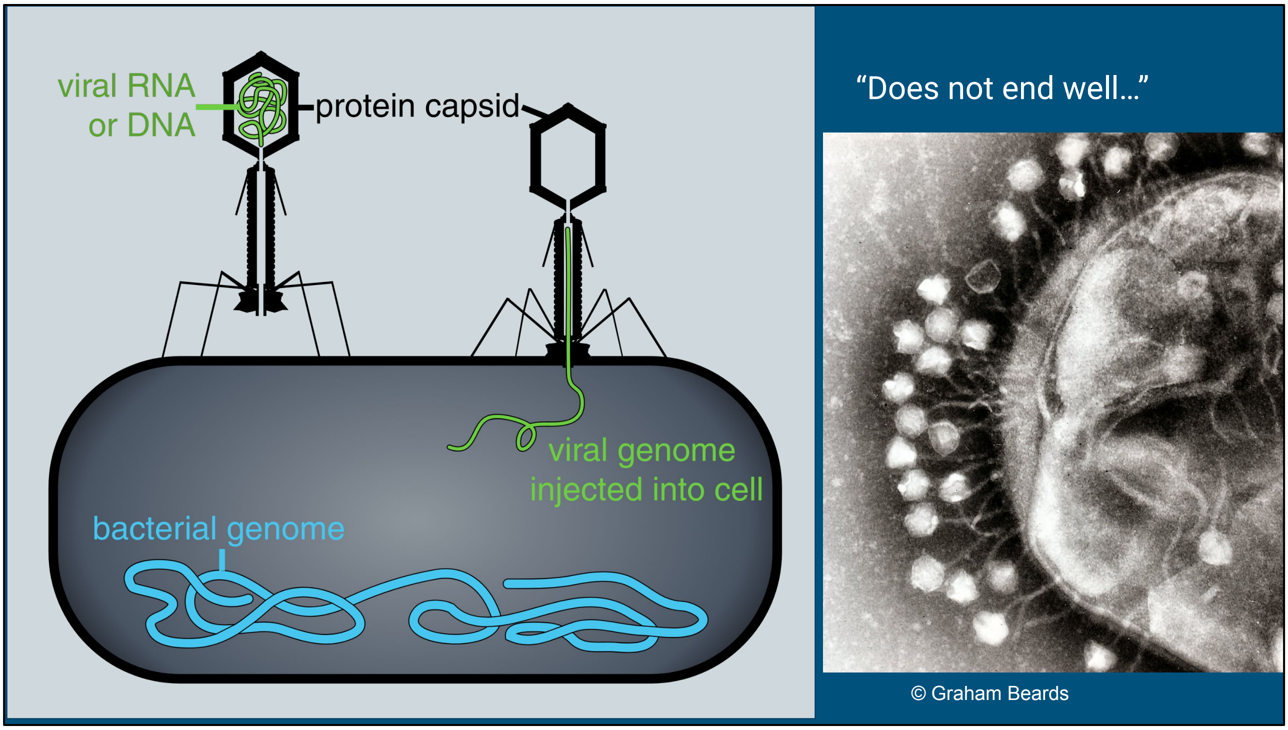


Bacteria have viruses too..

T4

Dr. Victor Padilla-Sanchez, PhD https://www.drvictorpadillasanchez.com

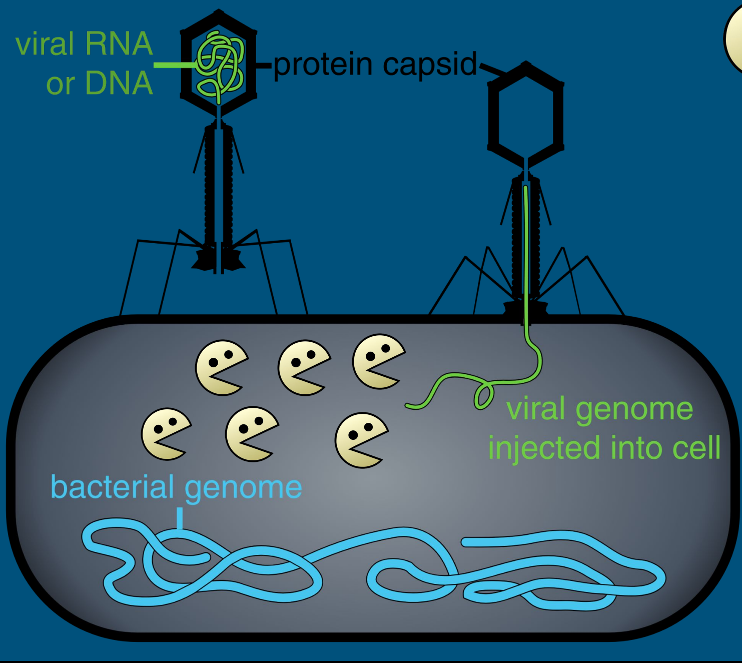
https://en.wikipedia.org/wiki/Bacteriophage#/media/File:PhageExterior.svg
http://stl-bjb.ac-dijon.fr/spip.php?article32
https://en.wikipedia.org/wiki/Escherichia_virus_T4
https://commons.wikimedia.org/wiki/File:Bacteriophage_T4_Structural_Model_at_Atomic_Resolution.tif?page=1



"Does not end well…"

© Graham Beards

By Professor Graham Beards - en:Image:Phage.jpg, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=5035798 https://commons.wikimedia.org/wiki/File:Phage_injecting_its_genome_into_bacteria.svg



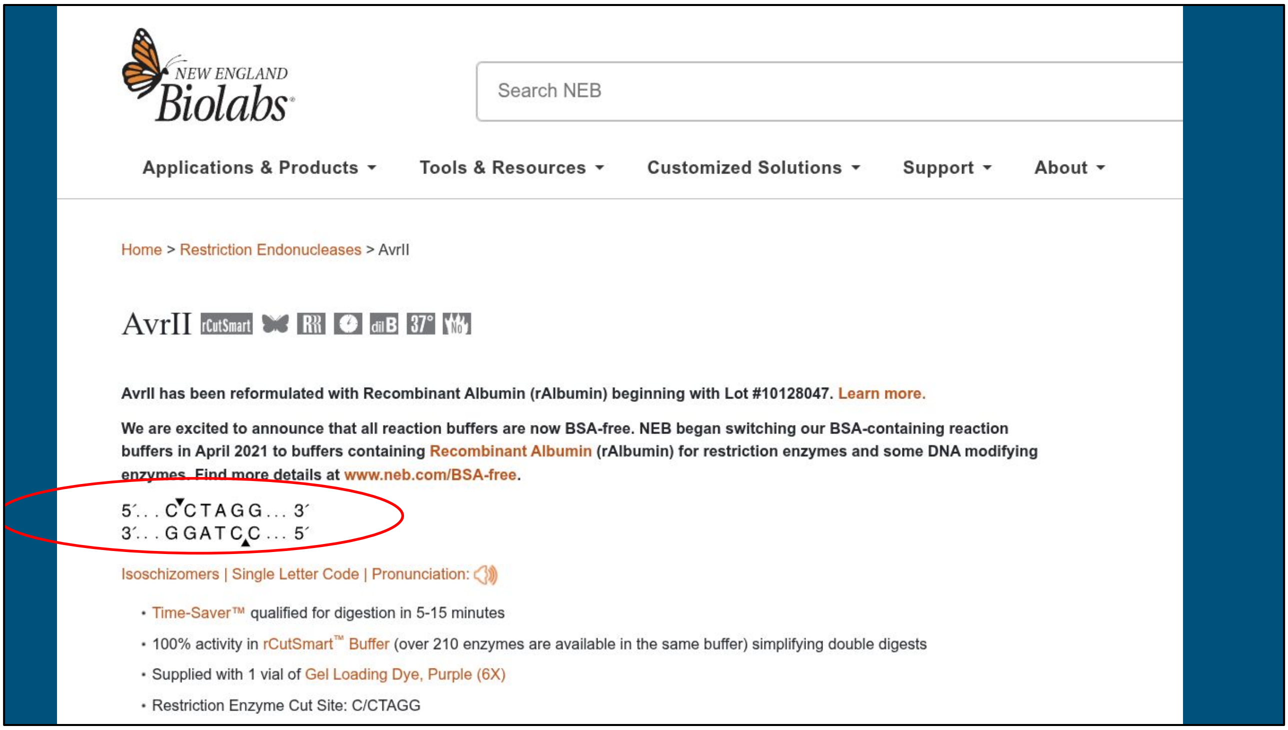
ᗧ ♥ CCTAGG

2^{-12} = 1 in every 4096 DNA characters at random… SNIP

This **restricts** the attacking DNA

Bacteria apply **modifications** to their own CCTAGG instances for protection

Not perfect though… so they avoid the sequence themselves



Home > Restriction Endonucleases > AvrII

AvrII

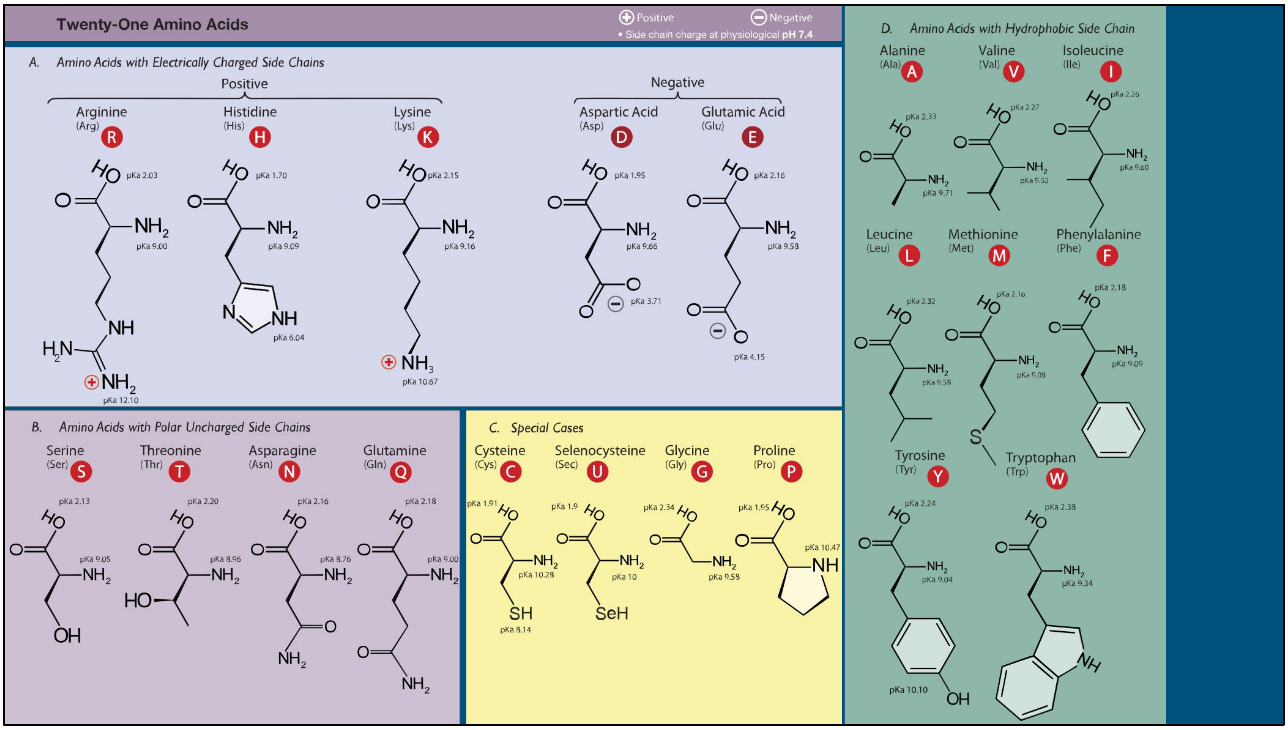
AvrII has been reformulated with Recombinant Albumin (rAlbumin) beginning with Lot #10128047. Learn more.

We are excited to announce that all reaction buffers are now BSA-free. NEB began switching our BSA-containing reaction buffers in April 2021 to buffers containing Recombinant Albumin (rAlbumin) for restriction enzymes and some DNA modifying enzymes. Find more details at www.neb.com/BSA-free.

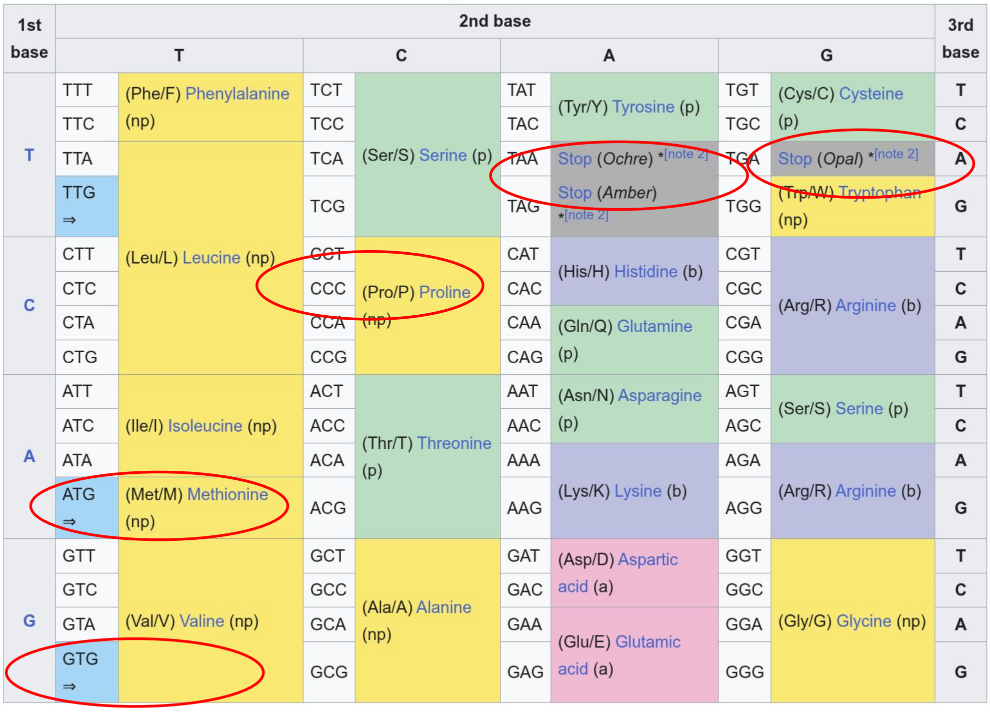
5´. . . C▼CTAGG . . . 3´
3´. . . GGATC▲C . . . 5´

Isoschizomers | Single Letter Code | Pronunciation:

- Time-Saver™ qualified for digestion in 5-15 minutes
- 100% activity in rCutSmart™ Buffer (over 210 enzymes are available in the same buffer) simplifying double digests
- Supplied with 1 vial of Gel Loading Dye, Purple (6X)
- Restriction Enzyme Cut Site: C/CTAGG



Modified from https://commons.wikimedia.org/wiki/File:Amino_Acids.svg

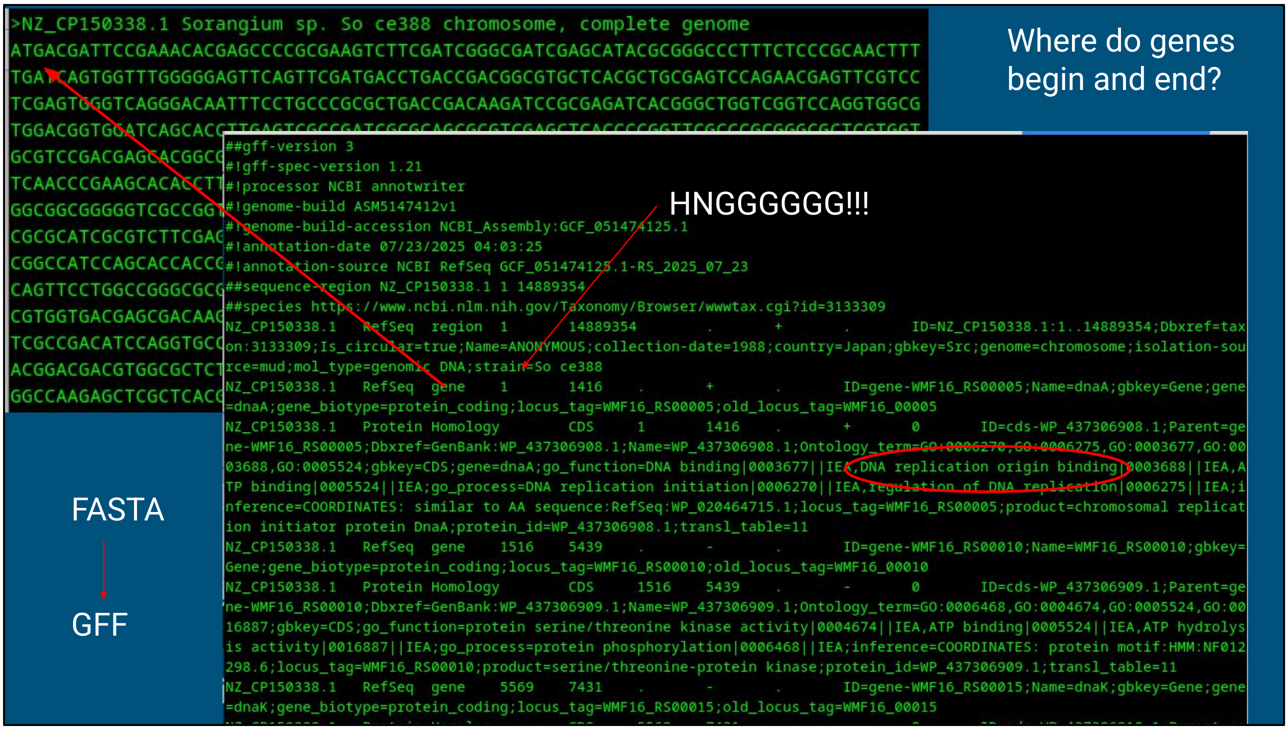


1st base	2nd base								3rd base
	T		C		A		G		
T	TTT	(Phe/F) Phenylalanine (np)	TCT	(Ser/S) Serine (p)	TAT	(Tyr/Y) Tyrosine (p)	TGT	(Cys/C) Cysteine (p)	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucine (np)	TCA		TAA	Stop (*Ochre*) *[note 2]	TGA	Stop (*Opal*) *[note 2]	A
	TTG ⇒		TCG		TAG	Stop (*Amber*) *[note 2]	TGG	(Trp/W) Tryptophan (np)	G
C	CTT		CCT	(Pro/P) Proline (np)	CAT	(His/H) Histidine (b)	CGT	(Arg/R) Arginine (b)	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutamine (p)	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucine (np)	ACT	(Thr/T) Threonine (p)	AAT	(Asn/N) Asparagine (p)	AGT	(Ser/S) Serine (p)	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lysine (b)	AGA	(Arg/R) Arginine (b)	A
	ATG ⇒	(Met/M) Methionine (np)	ACG		AAG		AGG		G
G	GTT	(Val/V) Valine (np)	GCT	(Ala/A) Alanine (np)	GAT	(Asp/D) Aspartic acid (a)	GGT	(Gly/G) Glycine (np)	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Glutamic acid (a)	GGA		A
	GTG ⇒		GCG		GAG		GGG		G

Multi-billion year old table!

Multiple codons for same amino acids

This allows for **dialects** and shaping DNA



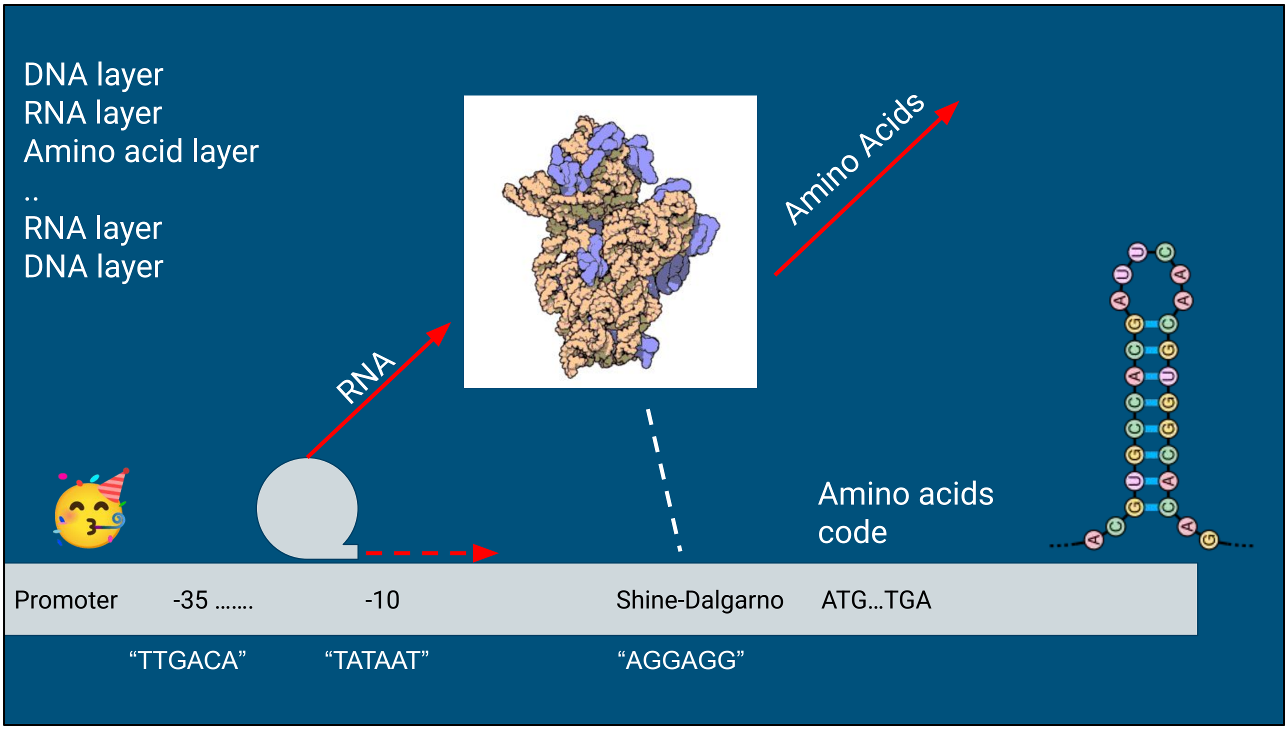
Where do genes begin and end?

```
>NZ_CP150338.1 Sorangium sp. So ce388 chromosome, complete genome
ATGACGATTCCGAAACACGAGCCCCGCGAAGTCTTCGATCGGGCGATCGAGCATACGCGGGCCCTTTCTCCCGCAACTTT
TGATCAGTGGTTTGGGGGAGTTCAGTTCGATGACCTGACCGACGGCGTGCTCACGCTGCGAGTCCAGAACGAGTTCGTCC
TCGAGTGGGTCAGGGACAATTTCCTGCCCGCGCTGACCGACAAGATCCGCGAGATCACGGGCTGGTCGGTCCAGGTGGCG
TGGACGGTGGATCAGCACC
GCGTCCGACGAGCACGGCG
TCAACCCGAAGCACACCTT
GGCGGCGGGGGTCGCCGGT
CGCGCATCGCGTCTTCGAG
CGGCCATCCAGCACCACCG
CAGTTCCTGGCCGGGCGCG
CGTGGTGACGAGCGACAAG
TCGCCGACATCCAGGTGCC
ACGGACGACGTGGCGCTCT
GGCCAAGAGCTCGCTCACG
```

```
##gff-version 3
#!gff-spec-version 1.21
#!processor NCBI annotwriter
#!genome-build ASM5147412v1
#!genome-build-accession NCBI_Assembly:GCF_051474125.1
#!annotation-date 07/23/2025 04:03:25
#!annotation-source NCBI RefSeq GCF_051474125.1-RS_2025_07_23
##sequence-region NZ_CP150338.1 1 14889354
##species https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=3133309
NZ_CP150338.1   RefSeq  region  1       14889354        .       +       .       ID=NZ_CP150338.1:1..14889354;Dbxref=taxon:3133309;Is_circular=true;Name=ANONYMOUS;collection-date=1988;country=Japan;gbkey=Src;genome=chromosome;isolation-source=mud;mol_type=genomic DNA;strain=So ce388
NZ_CP150338.1   RefSeq  gene    1       1416    .       +       .       ID=gene-WMF16_RS00005;Name=dnaA;gbkey=Gene;gene=dnaA;gene_biotype=protein_coding;locus_tag=WMF16_RS00005;old_locus_tag=WMF16_00005
NZ_CP150338.1   Protein Homology        CDS     1       1416    .       +       0       ID=cds-WP_437306908.1;Parent=gene-WMF16_RS00005;Dbxref=GenBank:WP_437306908.1;Name=WP_437306908.1;Ontology_term=GO:0006270,GO:0006275,GO:0003677,GO:0003688,GO:0005524;gbkey=CDS;gene=dnaA;go_function=DNA binding|0003677||IEA,DNA replication origin binding|0003688||IEA,ATP binding|0005524||IEA;go_process=DNA replication initiation|0006270||IEA,regulation of DNA replication|0006275||IEA;inference=COORDINATES: similar to AA sequence:RefSeq:WP_020464715.1;locus_tag=WMF16_RS00005;product=chromosomal replication initiator protein DnaA;protein_id=WP_437306908.1;transl_table=11
NZ_CP150338.1   RefSeq  gene    1516    5439    .       -       .       ID=gene-WMF16_RS00010;Name=WMF16_RS00010;gbkey=Gene;gene_biotype=protein_coding;locus_tag=WMF16_RS00010;old_locus_tag=WMF16_00010
NZ_CP150338.1   Protein Homology        CDS     1516    5439    .       -       0       ID=cds-WP_437306909.1;Parent=gene-WMF16_RS00010;Dbxref=GenBank:WP_437306909.1;Name=WP_437306909.1;Ontology_term=GO:0006468,GO:0004674,GO:0005524,GO:0016887;gbkey=CDS;go_function=protein serine/threonine kinase activity|0004674||IEA,ATP binding|0005524||IEA,ATP hydrolysis activity|0016887||IEA;go_process=protein phosphorylation|0006468||IEA;inference=COORDINATES: protein motif:HMM:NF012298.6;locus_tag=WMF16_RS00010;product=serine/threonine-protein kinase;protein_id=WP_437306909.1;transl_table=11
NZ_CP150338.1   RefSeq  gene    5569    7431    .       -       .       ID=gene-WMF16_RS00015;Name=dnaK;gbkey=Gene;gene=dnaK;gene_biotype=protein_coding;locus_tag=WMF16_RS00015;old_locus_tag=WMF16_00015
```

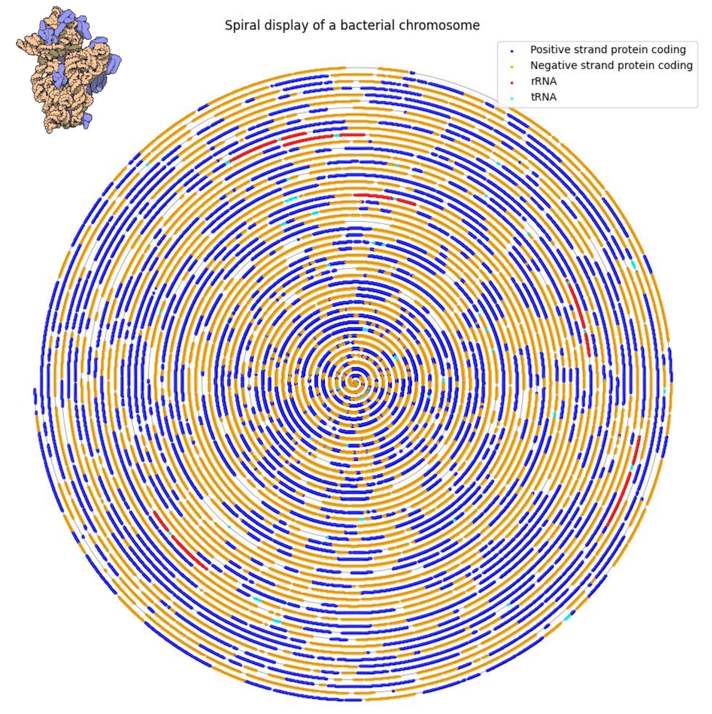
HNGGGGGG!!!

FASTA → GFF



DNA layer
RNA layer
Amino acid layer
..
RNA layer
DNA layer

https://www.biorxiv.org/content/10.1101/2025.01.23.634641v2.full



Escherichia coli str. K-12 substr. W3110

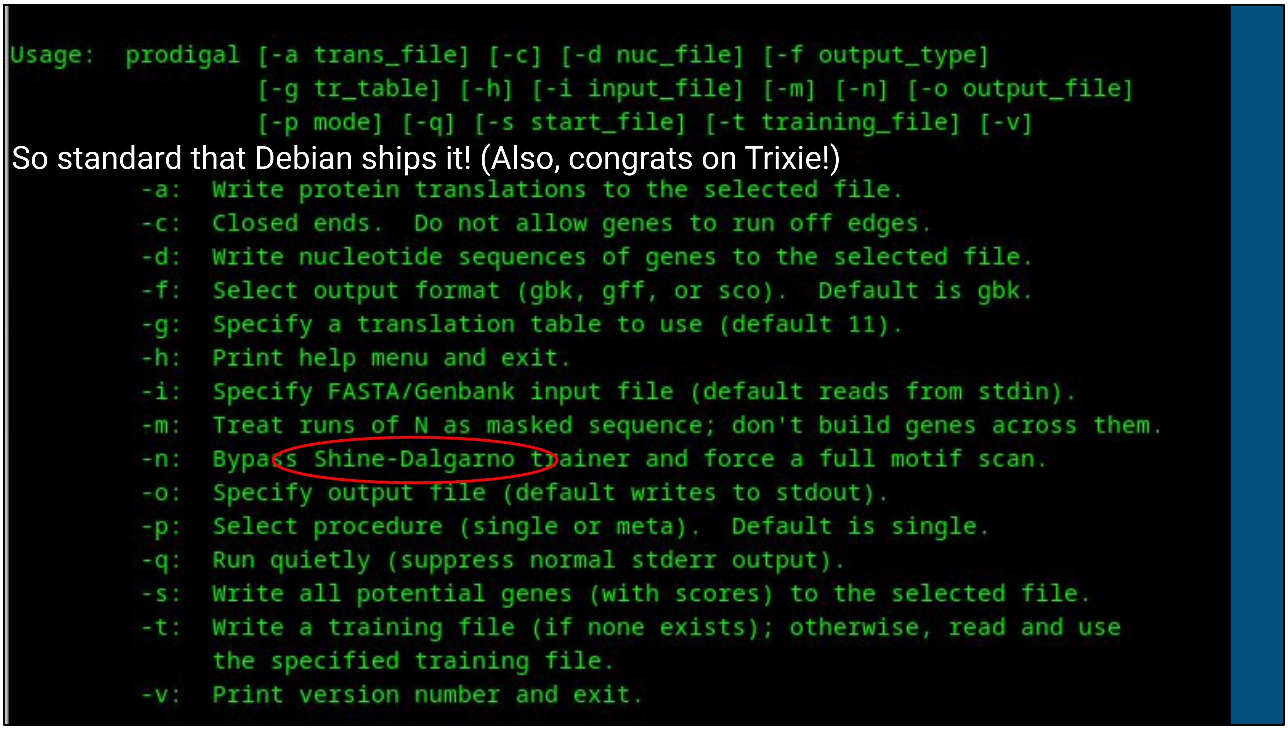
Topologically this chromosome is actually a circle.

Spiral shape however is better for an overview

The blank areas are **not genes**. Partially we know what this is. Partially not!

Biologists study genes.. “Looking for your keys where the light is”

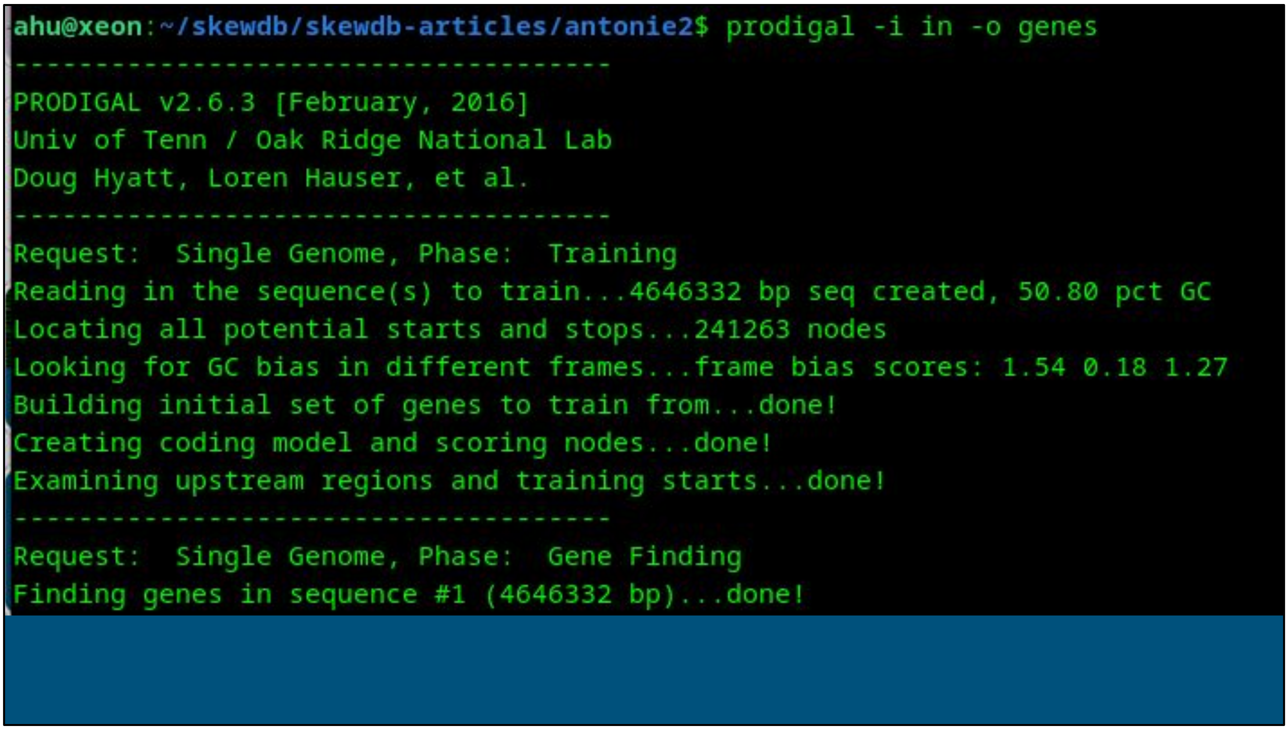
But us nerds could take a look at the data!



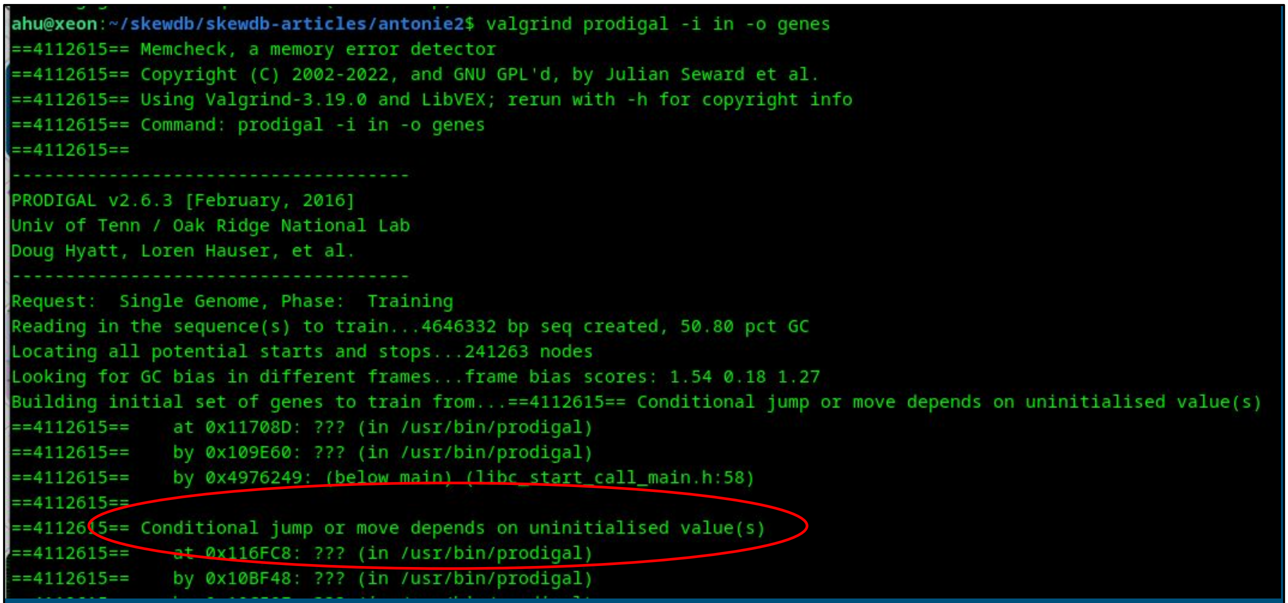
So standard that Debian ships it! (Also, congrats on Trixie!)

```
Usage:  prodigal [-a trans_file] [-c] [-d nuc_file] [-f output_type]
                 [-g tr_table] [-h] [-i input_file] [-m] [-n] [-o output_file]
                 [-p mode] [-q] [-s start_file] [-t training_file] [-v]

         -a:  Write protein translations to the selected file.
         -c:  Closed ends.  Do not allow genes to run off edges.
         -d:  Write nucleotide sequences of genes to the selected file.
         -f:  Select output format (gbk, gff, or sco).  Default is gbk.
         -g:  Specify a translation table to use (default 11).
         -h:  Print help menu and exit.
         -i:  Specify FASTA/Genbank input file (default reads from stdin).
         -m:  Treat runs of N as masked sequence; don't build genes across them.
         -n:  Bypass Shine-Dalgarno trainer and force a full motif scan.
         -o:  Specify output file (default writes to stdout).
         -p:  Select procedure (single or meta).  Default is single.
         -q:  Run quietly (suppress normal stderr output).
         -s:  Write all potential genes (with scores) to the selected file.
         -t:  Write a training file (if none exists); otherwise, read and use
              the specified training file.
         -v:  Print version number and exit.
```

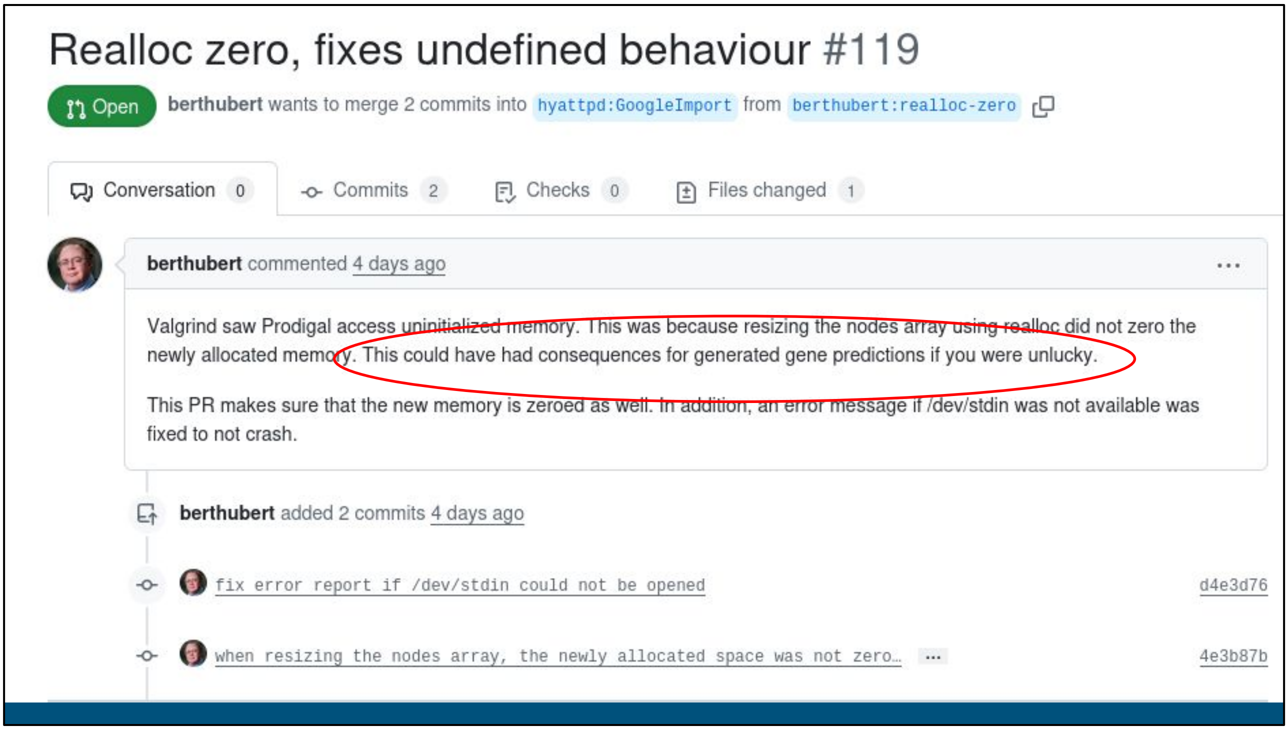


```
ahu@xeon:~/skewdb/skewdb-articles/antonie2$ prodigal -i in -o genes
-------------------------------------
PRODIGAL v2.6.3 [February, 2016]
Univ of Tenn / Oak Ridge National Lab
Doug Hyatt, Loren Hauser, et al.
-------------------------------------
Request:  Single Genome, Phase:  Training
Reading in the sequence(s) to train...4646332 bp seq created, 50.80 pct GC
Locating all potential starts and stops...241263 nodes
Looking for GC bias in different frames...frame bias scores: 1.54 0.18 1.27
Building initial set of genes to train from...done!
Creating coding model and scoring nodes...done!
Examining upstream regions and training starts...done!
-------------------------------------
Request:  Single Genome, Phase:  Gene Finding
Finding genes in sequence #1 (4646332 bp)...done!
```



```
ahu@xeon:~/skewdb/skewdb-articles/antonie2$ valgrind prodigal -i in -o genes
==4112615== Memcheck, a memory error detector
==4112615== Copyright (C) 2002-2022, and GNU GPL'd, by Julian Seward et al.
==4112615== Using Valgrind-3.19.0 and LibVEX; rerun with -h for copyright info
==4112615== Command: prodigal -i in -o genes
==4112615==
-------------------------------------
PRODIGAL v2.6.3 [February, 2016]
Univ of Tenn / Oak Ridge National Lab
Doug Hyatt, Loren Hauser, et al.
-------------------------------------
Request:  Single Genome, Phase:  Training
Reading in the sequence(s) to train...4646332 bp seq created, 50.80 pct GC
Locating all potential starts and stops...241263 nodes
Looking for GC bias in different frames...frame bias scores: 1.54 0.18 1.27
Building initial set of genes to train from...==4112615== Conditional jump or move depends on uninitialised value(s)
==4112615==    at 0x11708D: ??? (in /usr/bin/prodigal)
==4112615==    by 0x109E60: ??? (in /usr/bin/prodigal)
==4112615==    by 0x4976249: (below main) (libc_start_call_main.h:58)
==4112615==
==4112615== Conditional jump or move depends on uninitialised value(s)
==4112615==    at 0x116FC8: ??? (in /usr/bin/prodigal)
==4112615==    by 0x10BF48: ??? (in /usr/bin/prodigal)
```

OOPS!!!!



Realloc zero, fixes undefined behaviour #119

Open **berthubert** wants to merge 2 commits into `hyattpd:GoogleImport` from `berthubert:realloc-zero`

berthubert commented 4 days ago

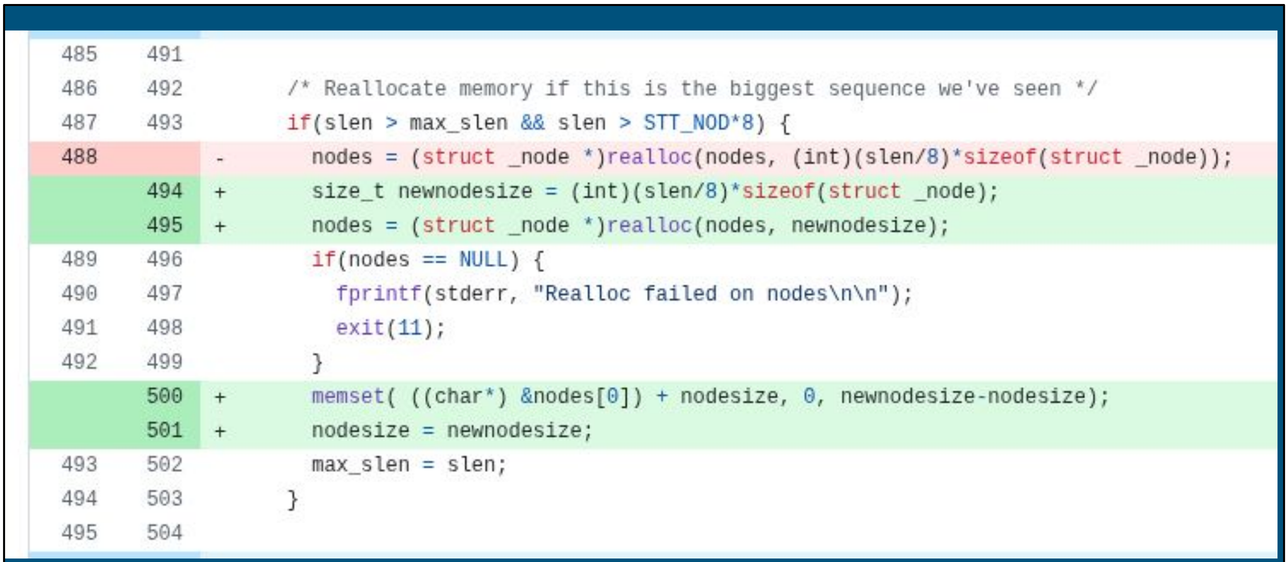
Valgrind saw Prodigal access uninitialized memory. This was because resizing the nodes array using realloc did not zero the newly allocated memory. This could have had consequences for generated gene predictions if you were unlucky.

This PR makes sure that the new memory is zeroed as well. In addition, an error message if /dev/stdin was not available was fixed to not crash.

berthubert added 2 commits 4 days ago

`fix error report if /dev/stdin could not be opened` d4e3d76

`when resizing the nodes array, the newly allocated space was not zero…` 4e3b87b



```
  /* Reallocate memory if this is the biggest sequence we've seen */
  if(slen > max_slen && slen > STT_NOD*8) {
-   nodes = (struct _node *)realloc(nodes, (int)(slen/8)*sizeof(struct _node));
+   size_t newnodesize = (int)(slen/8)*sizeof(struct _node);
+   nodes = (struct _node *)realloc(nodes, newnodesize);
    if(nodes == NULL) {
      fprintf(stderr, "Realloc failed on nodes\n\n");
      exit(11);
    }
+   memset( ((char*) &nodes[0]) + nodesize, 0, newnodesize-nodesize);
+   nodesize = newnodesize;
    max_slen = slen;
  }

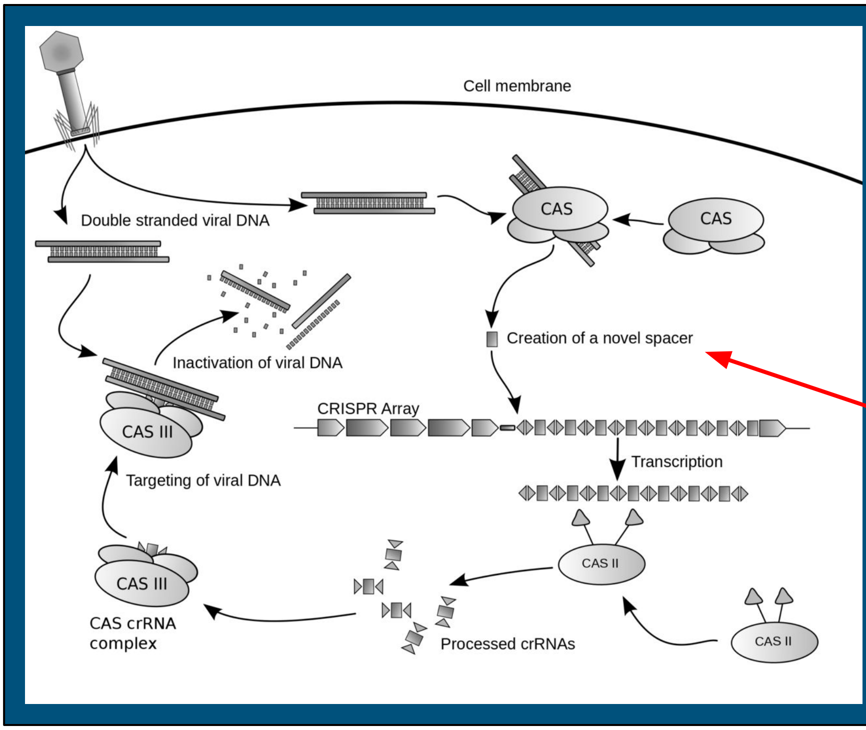
```

The state of bioinformatics software is… not great (second time this happened to me)

More bacterial anti-viral defenses.

This war has been raging for 2 billion years at least!

Maybe we could learn..



A multi-generational immune system.

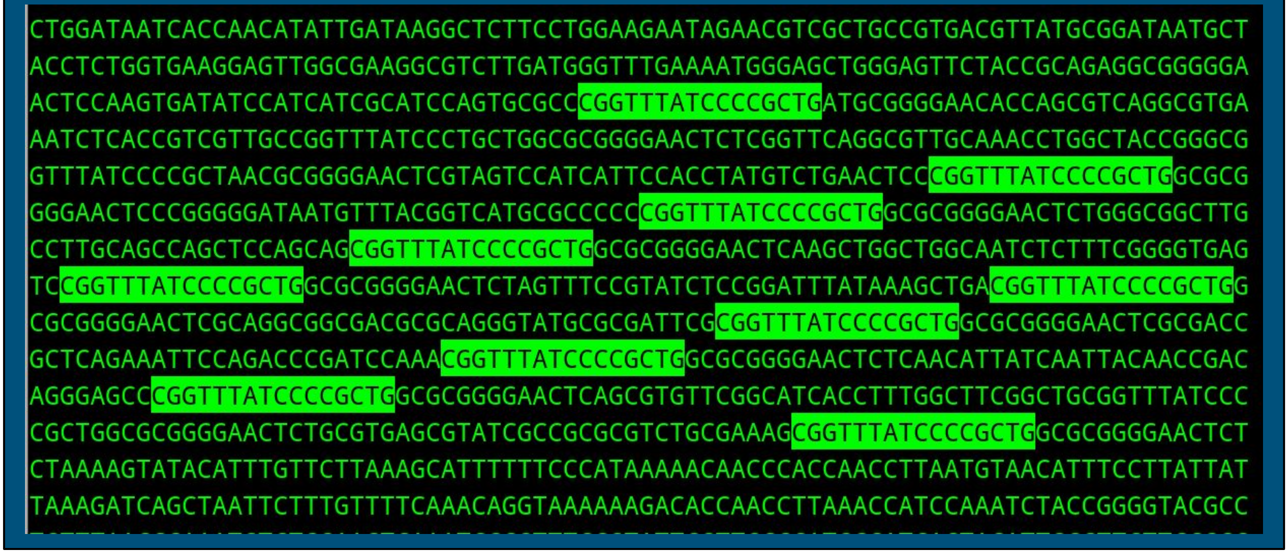
A forensic record of previously survived viruses!

Can we see it?

People looked at this for 20 years w/o knowing what it ws

By James atmos - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=7821536

CRISPR: “The origin of the spacer sequences remains unknown” - 2002



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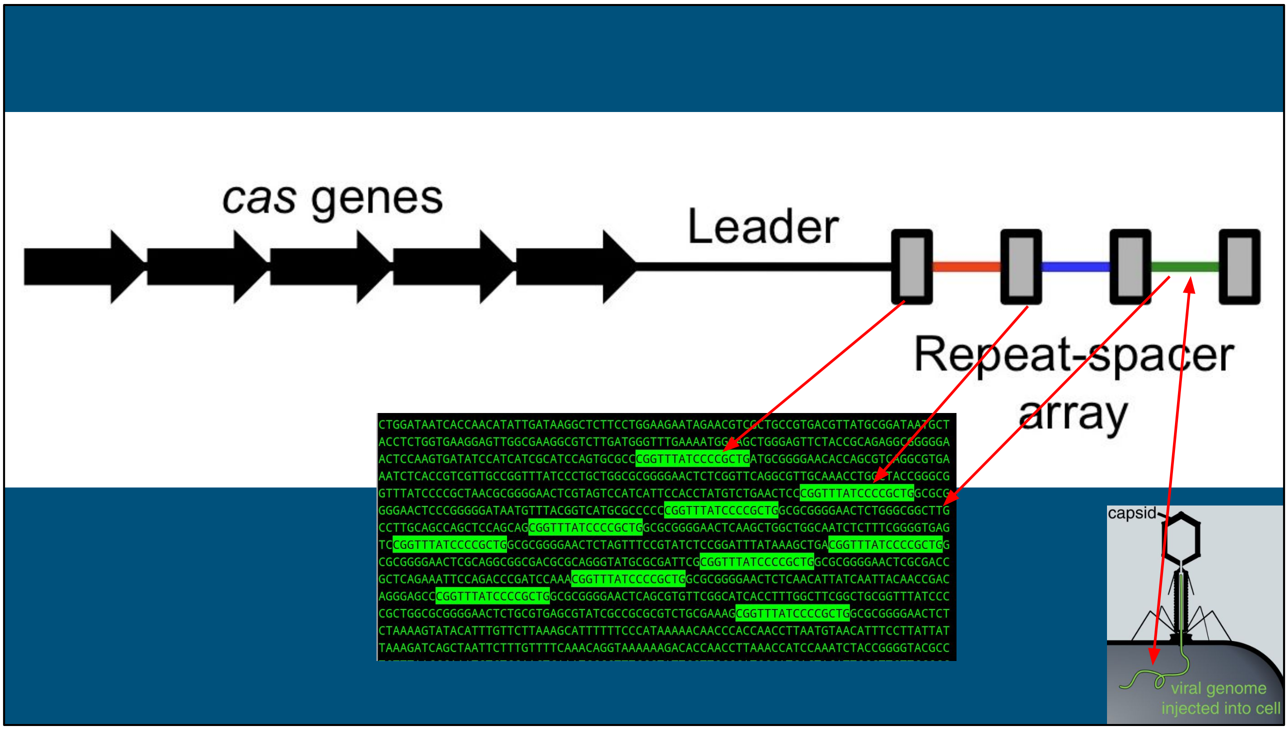
https://onlinelibrary.wiley.com/doi/epdf/10.1046/j.1365-2958.2002.02839.x



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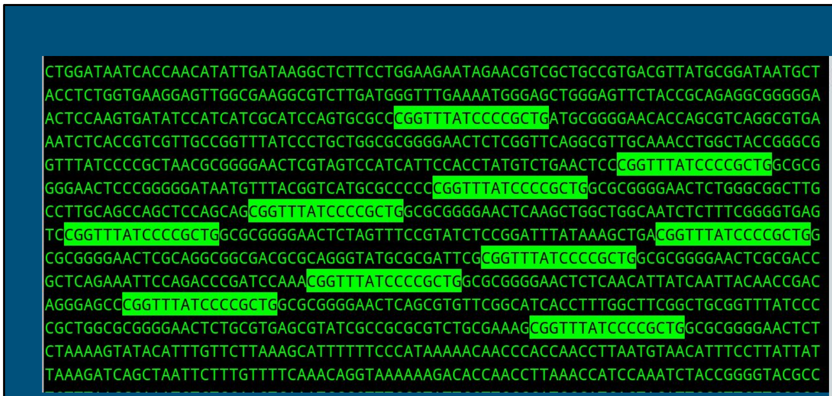

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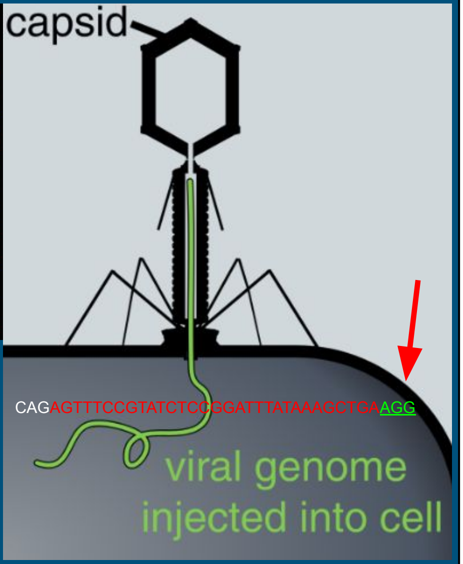
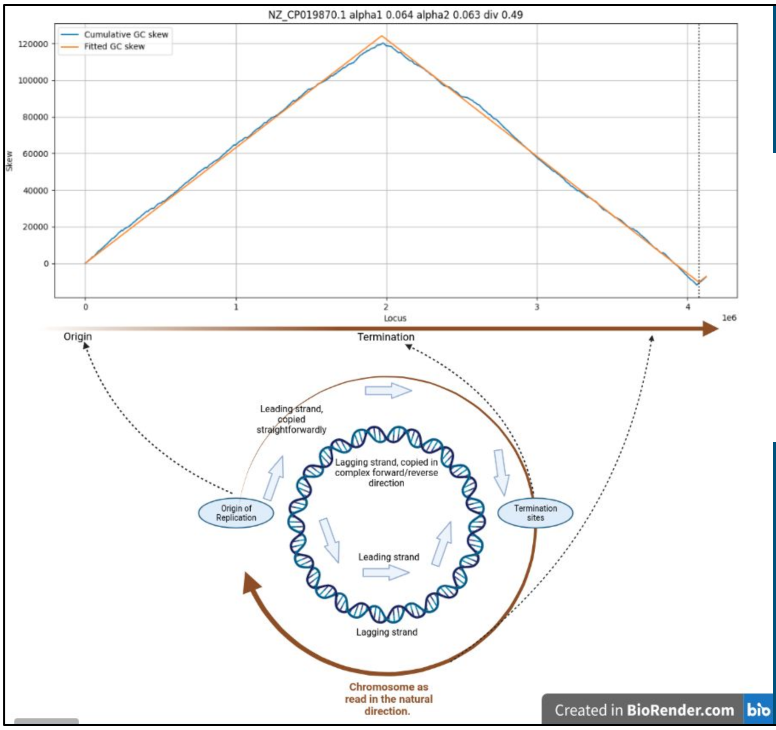


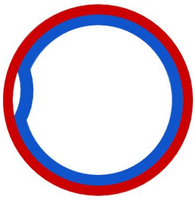
Signature:
"AGTTTCCGTATCTCCGGATTTATAAAGCTGA"

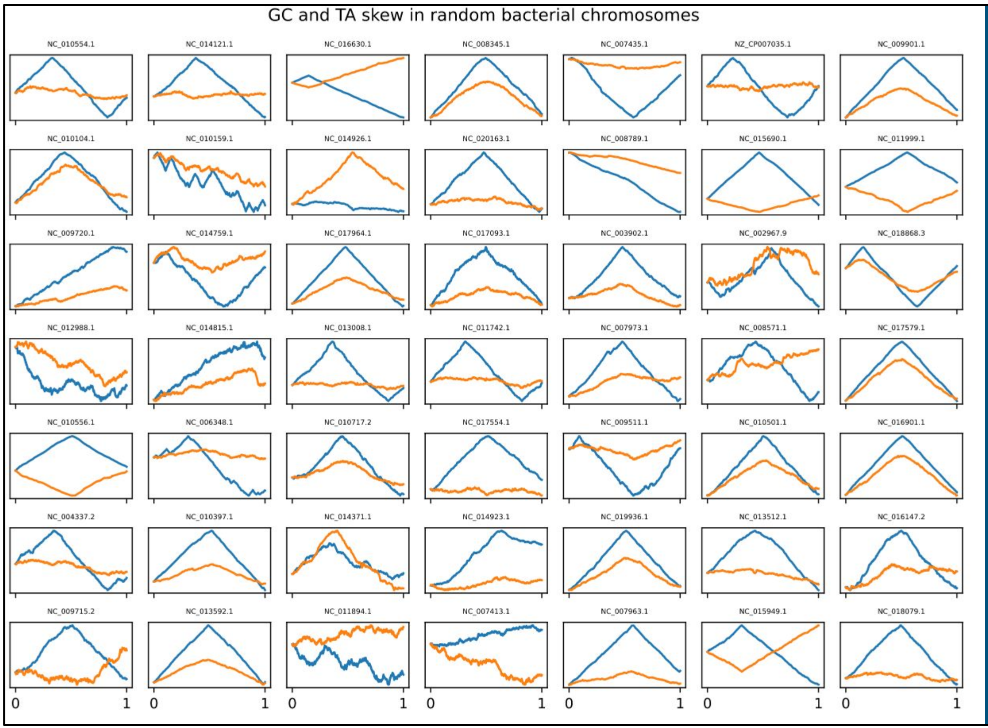
Why does the CRISPR system not destroy itself?



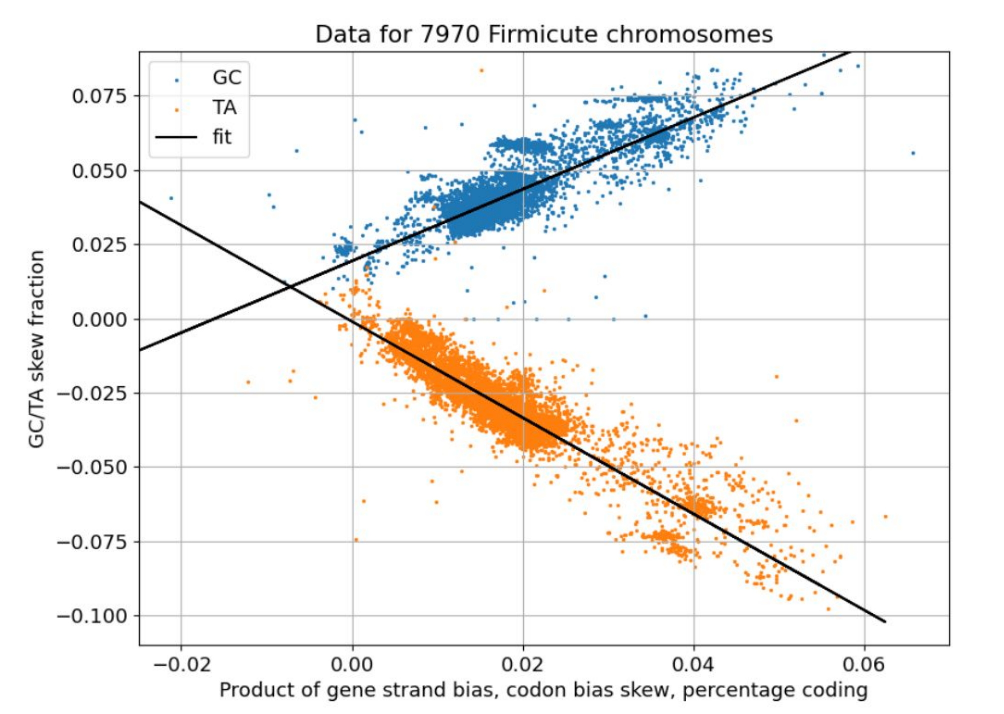
GC SKEW!

https://skewdb.org/view





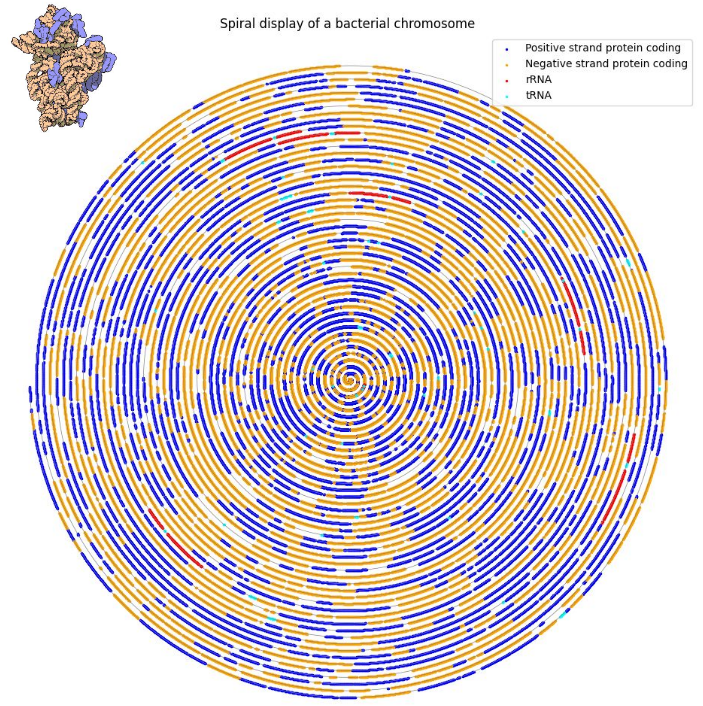
And no one really knows why!



Lots of data available to test hypotheses

I also have a few

Have a go with the data from skewdb.org!

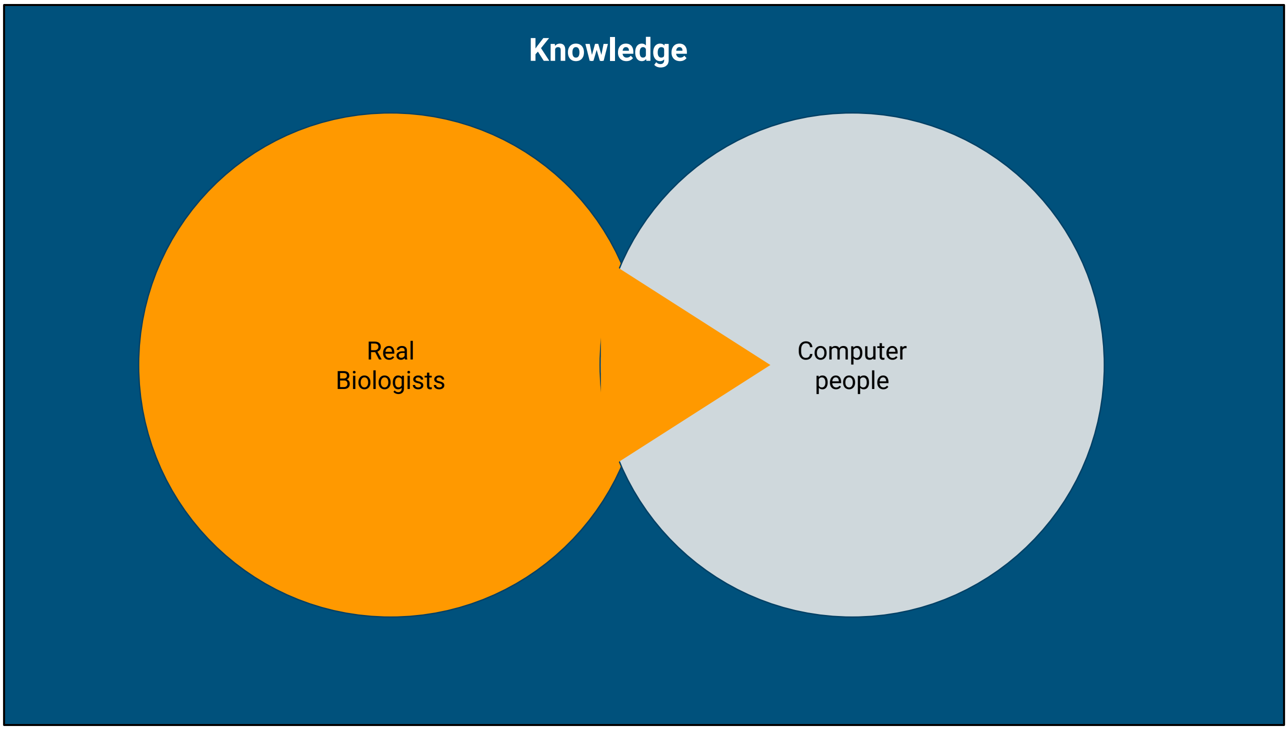


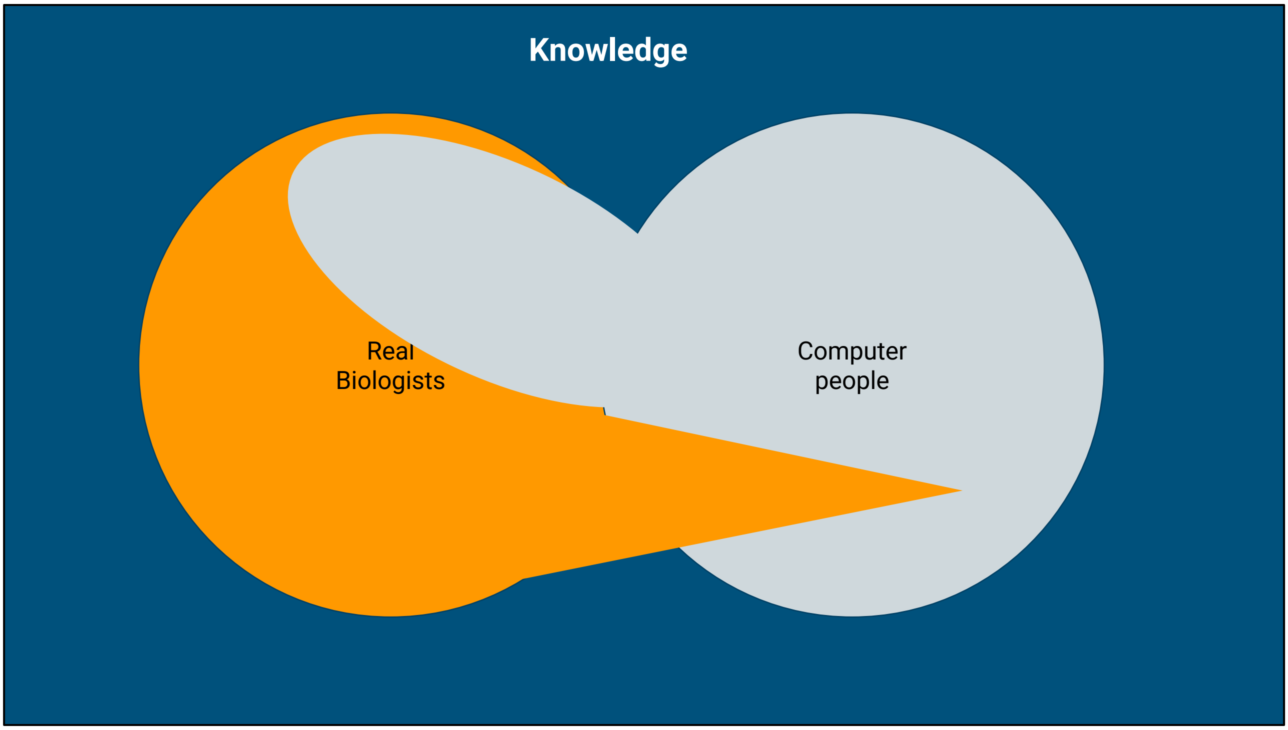
Escherichia coli str. K-12 substr. W3110

Look at the white spaces

Very interesting things could be hiding there

And you could help find out what it is!


Knowledge
Real Biologists
Computer people


Knowledge
Real Biologists
Computer people

Why does GC-skew exist?
Why even Chargaff’s 2nd rule?
What is in the bacterial whitespace?
Why is so much bioinformatics tooling so terrible?

GOOD LUCK!

Come to the afterparty!

Tomorrow, Monday, 2025-08-11 15:00–15:50, Cassiopeia

Interactive session, featuring all the skipped slides and lots of room for questions and answers!

https://berthub.eu/revdna/