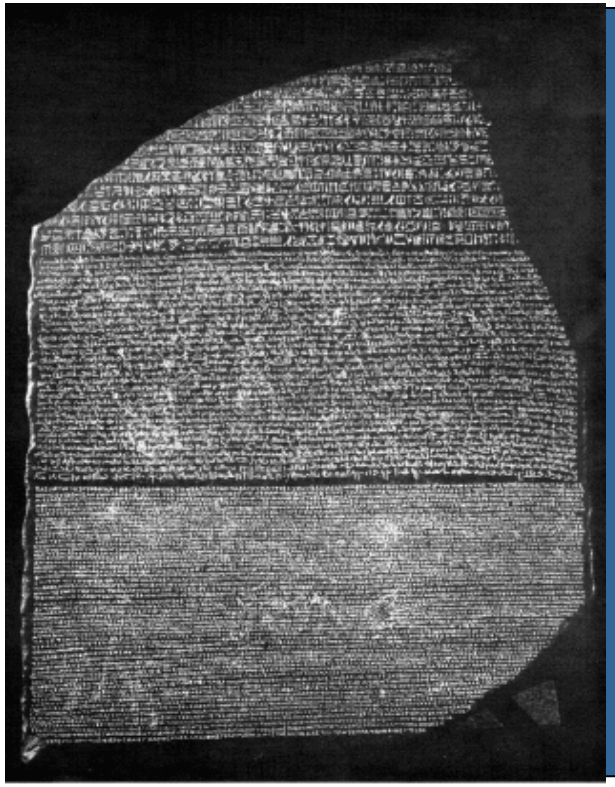

INTRODUCTION to COMPUTATIONAL GENOMICS

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Computational Genomics Lab

https://compgen.bio.ub.edu/

ESCI upf. School of International Studies

UNIVERSITAT DE BARCELONA

I→B=U→B

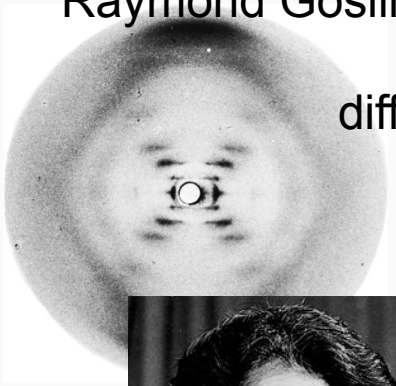
Summary

- Historical Context.
- Growth of Sequence Data.
- Sequencing in the Post-Genome Era.
- Sequence Annotation.
  - Basic Concepts.
  - Data Formats in CG.
  - Genomic Sequences Stats.
  - Sequences as Strings.

Index Medicus [1879]
National Library of Medicine (NLM)

Ada Lovelace first "program" [1843]
for Charles Babbage Analytical Engine

Raymond Gosling & Rosalind Franklin
DNA X-ray
diffraction pattern [1952]



Alan Turing's Machines [1936]

ENIAC [1943 – 1955†]
~18000 vacuum tubes
Electronic Numerical Integrator And Computer

James Watson &
Francis Crick **DNA**
structure model [1953]

High-level Programming Languages
FORTRAN [1957]

First protein sequence
bovine **insulin** [**1953**]

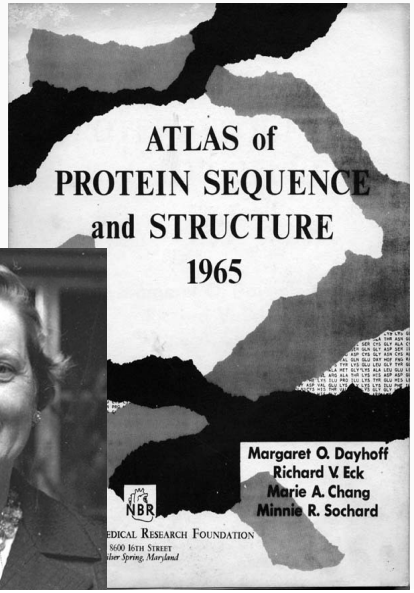
Structured Query Languages
SQL [1969]

ARPAnet [1969]

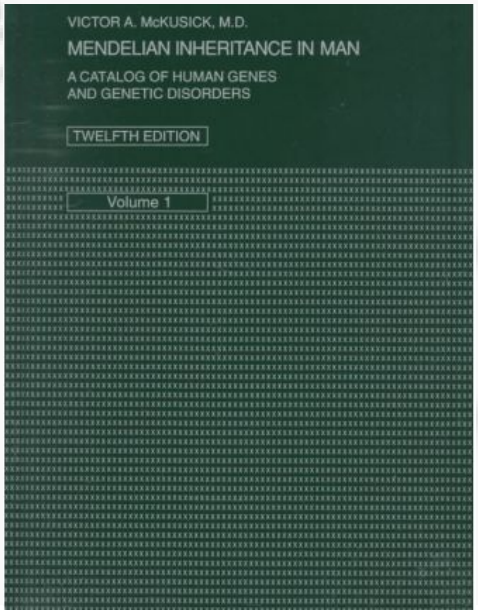
X-ray cristalography 3D structure
myoglobin protein [1959]

UNIX [1970]

Pioneering "Bioinformatics"
Margaret Dayhoff compiled
The Atlas of Protein Sequence and Structure
(~300 proteins) [**1965**], from those sequences
built first substitution matrices
(Point Accepted Mutations: PAM)



Dr. Victor A. McKusick
Mendelian Inheritance
in Man (MIM) [**1966**]
A catalog of mendelian
traits and disorders.



Protein Data Bank PDB [1971]
First database of protein structures

MEDLINE [1971]
Medical Literature Analysis
and Retrieval System Online

Maxam & Gilbert vs Sanger
DNA sequencing methods [**1975**]

First complete genome sequenced
Virus phi-X174 (~5400bp) [**1977**]

Global Alignment **Needleman-Wunsch** [1970]

Staden Package [1977]

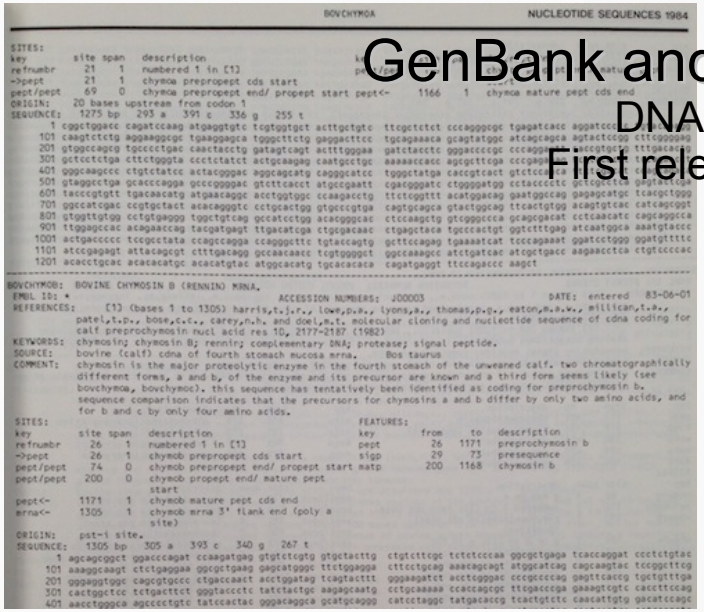
Local Alignment **Smith-Waterman** [1981]

First Personal Computer, IBM [1981]

GCG (Wisconsin Package) [1982]

Clustal alignment tool [1988]

Basic Local Alignment Search Tool
BLAST [Altschul, Gish, et al, 1990]


BOVCHYMOB NUCLEOTIDE SEQUENCES 1984

GenBank and EMBL [**1982**]
DNA sequence databases
First release of EMBL, june 1982,
contained **582 sequences**,
summing up to **600000bp**

Tim Berners-Lee
WWW [1991]

First Human Gene Sequenced
Huntington's disease [**1983**]

Kary B Mullis → **PCR** technique [1985]

Swiss-Prot [Amos Bairoch, **1986**]
Protein Sequence Database

National Center for Biotechnology Information (NCBI) [1988]

Human Genome Project Launched [**1990**]

Entrez [1991]
(NCBI's text search tool)
GenBank project
transitioned to NCBI [1992]
First bacterial genome sequenced
Haemophilus influenzae [**1995**]
Saccharomyces cerevisiae [**1996**]
PubMed (Public Medline) Launched [1997]
Caenorhabditis elegans genome [**1998**]
First human chromosome, chr22 [**1999**]
GEO and dbSNP [1999]
Drosophila melanogaster shotgun genome [**2000**]
Homo sapiens genome draft [**2001**]
Open Access Journals [PLoS, 2003]
Project ENCODE launched [2003]
DNA Origami [2006]
Illumina sequencing technology [2006]
Diploid genome of human single individuals [**2007**]
Synthetic bacterial genomes [2008]
Nanopore sequencing technology [1999/2014]
Synthetic yeast full genome [2017]
Earth BioGenome project [2018]
T2T: first complete, gapless sequence of a human genome [**2022**/**2023**]

Larry Page & Sergey Brin [1996]

genscan gene-finding program [1997]

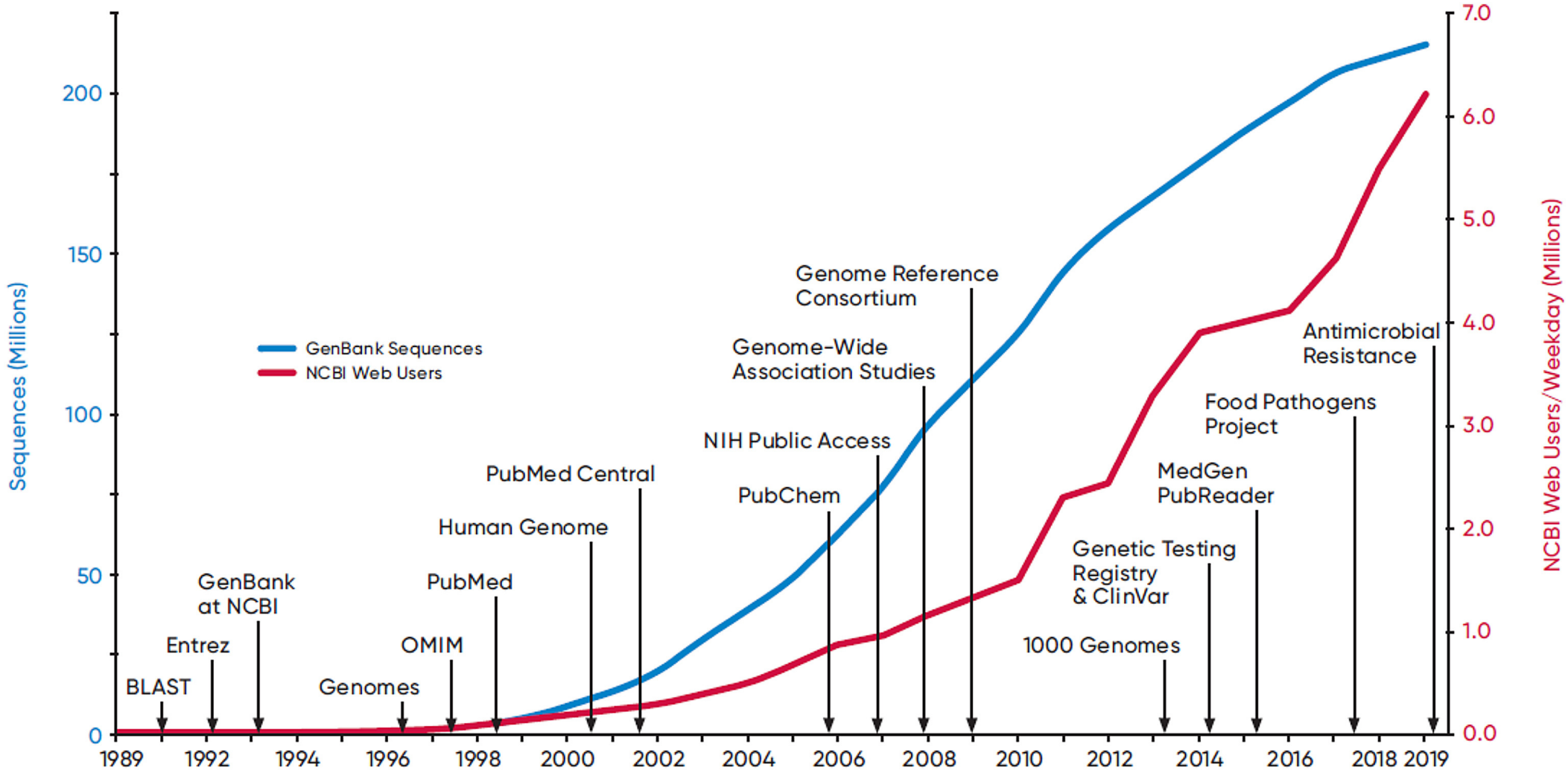
EMBOSS [1998]
European Molecular Biology
Open Software Suite

Genome Browsers
ENSEMBL & UCSC-GB
[2001/2002]

BioPython [2000]
BioConductor [2001]
BioPERL [2002]

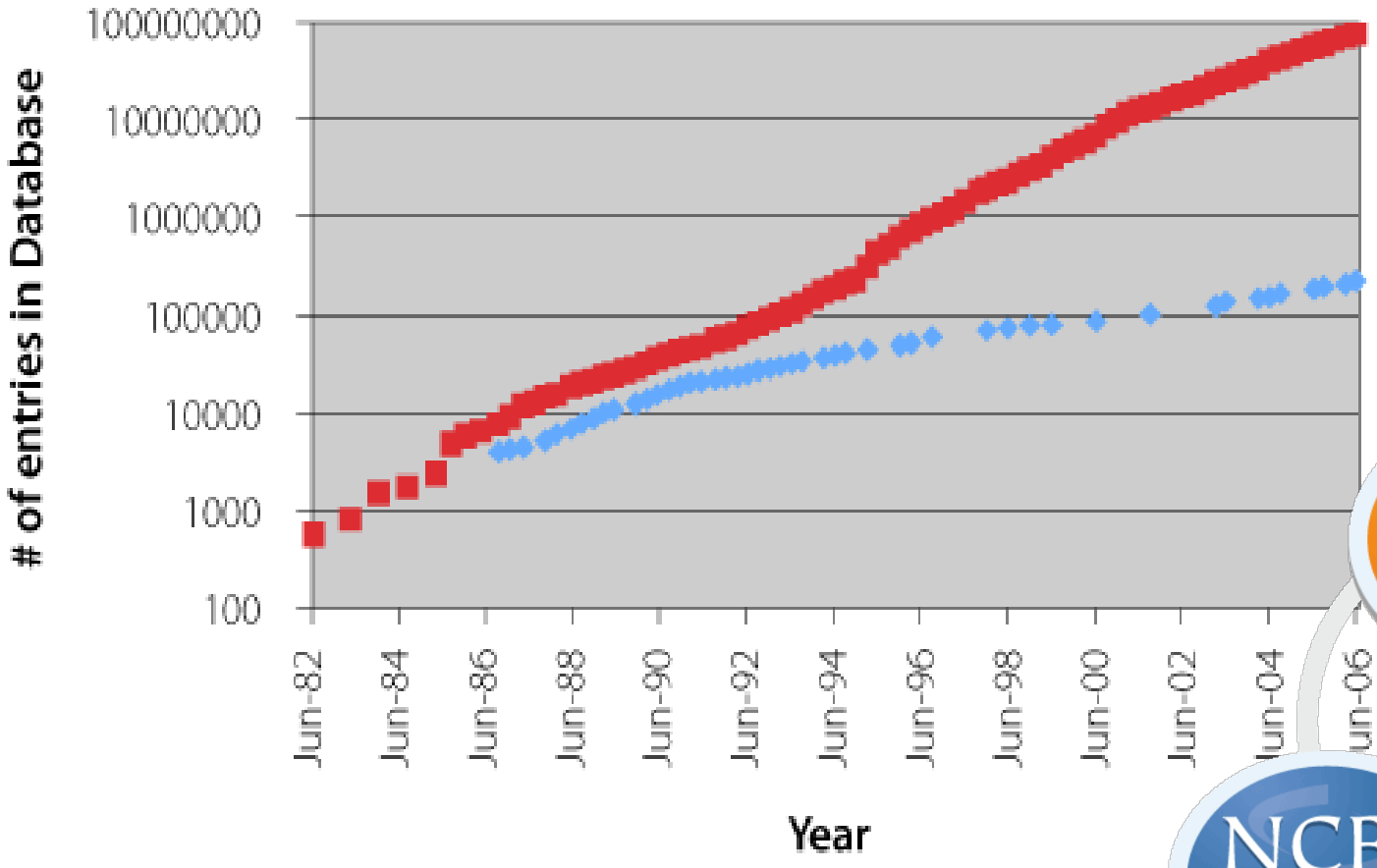
General AI
Deep Learning
& Big Data
[2012]

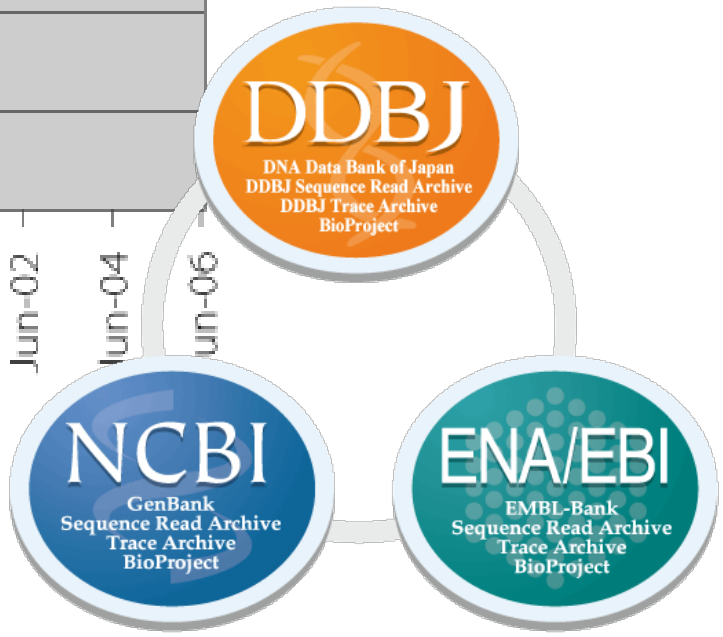
Growth of Sequence Databases



Growth of Sequence Databases Revisited

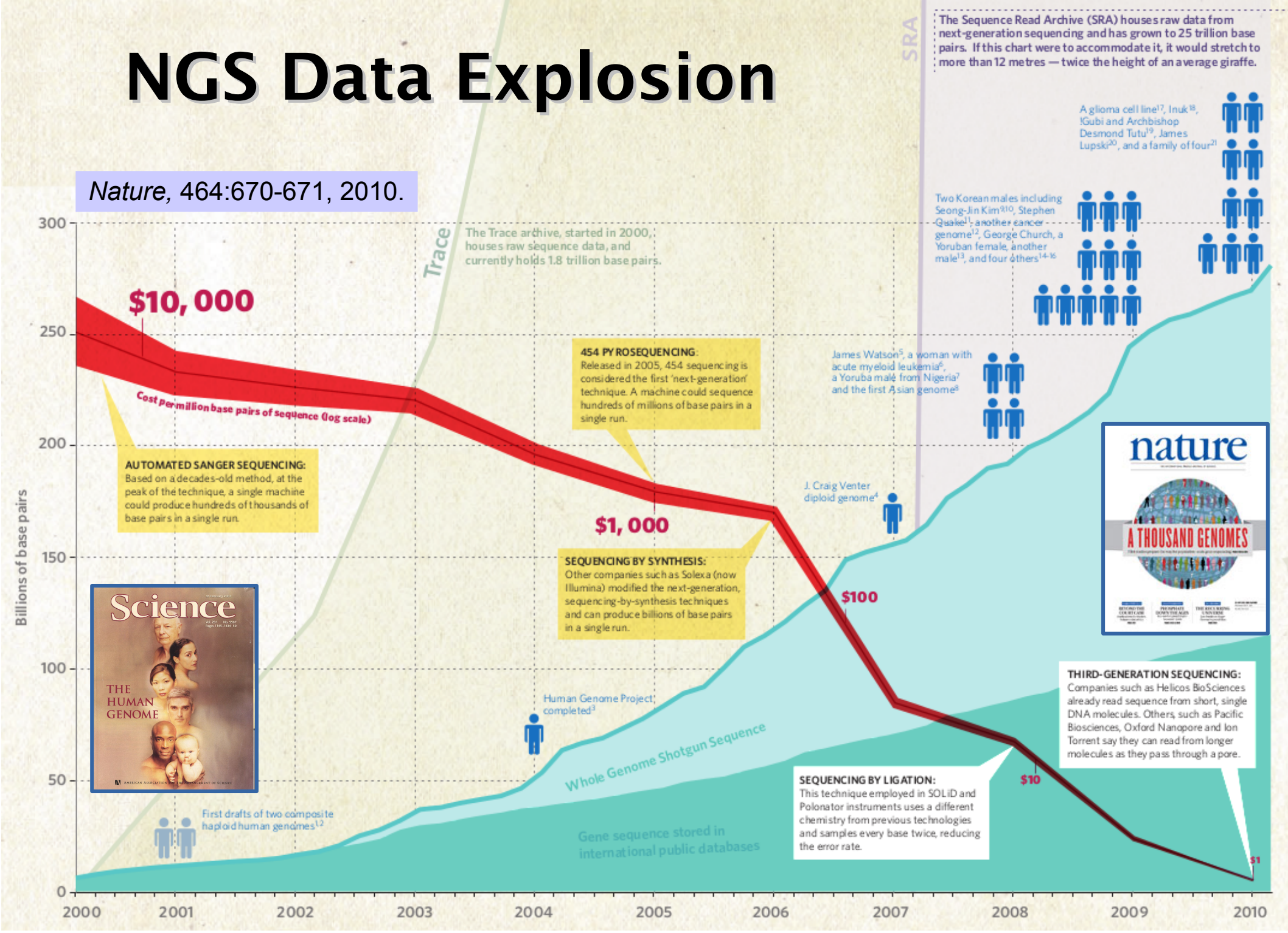
GenBank [*nucleotide*]



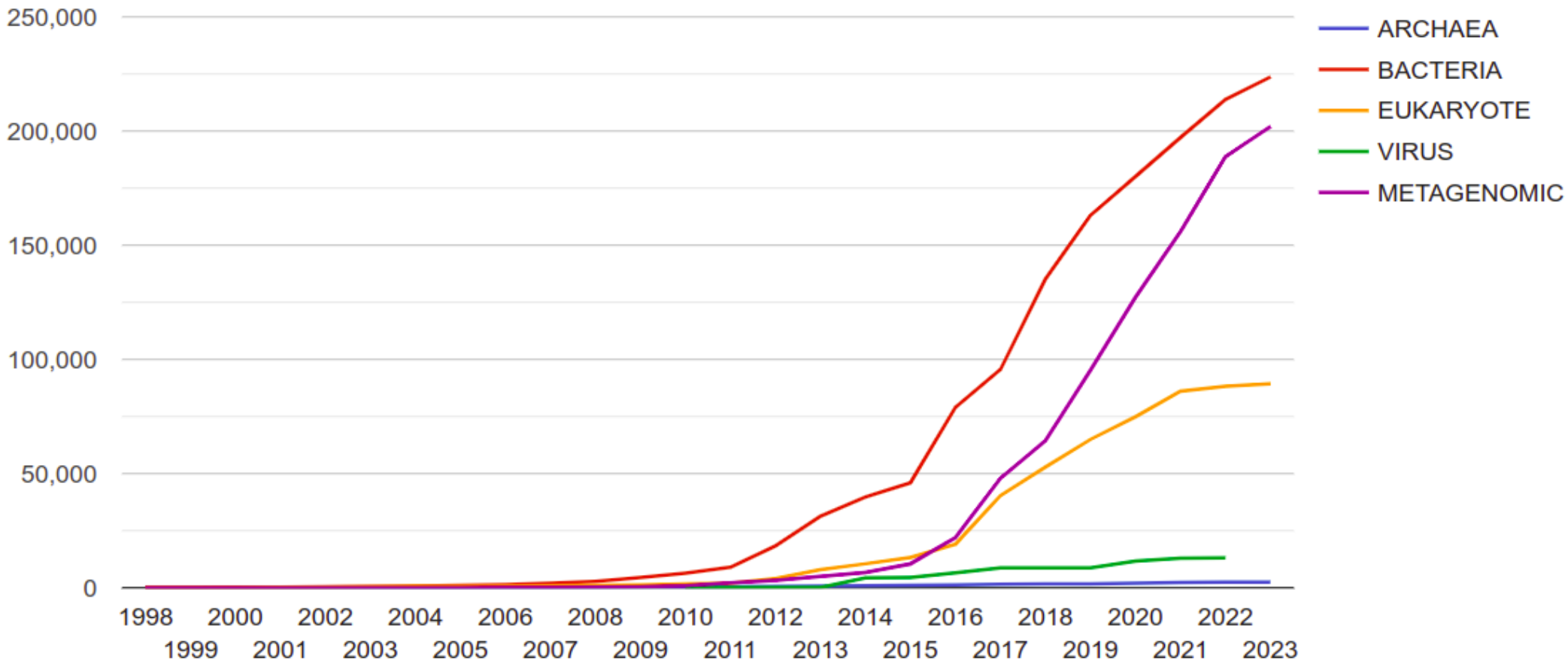


NGS Data Explosion

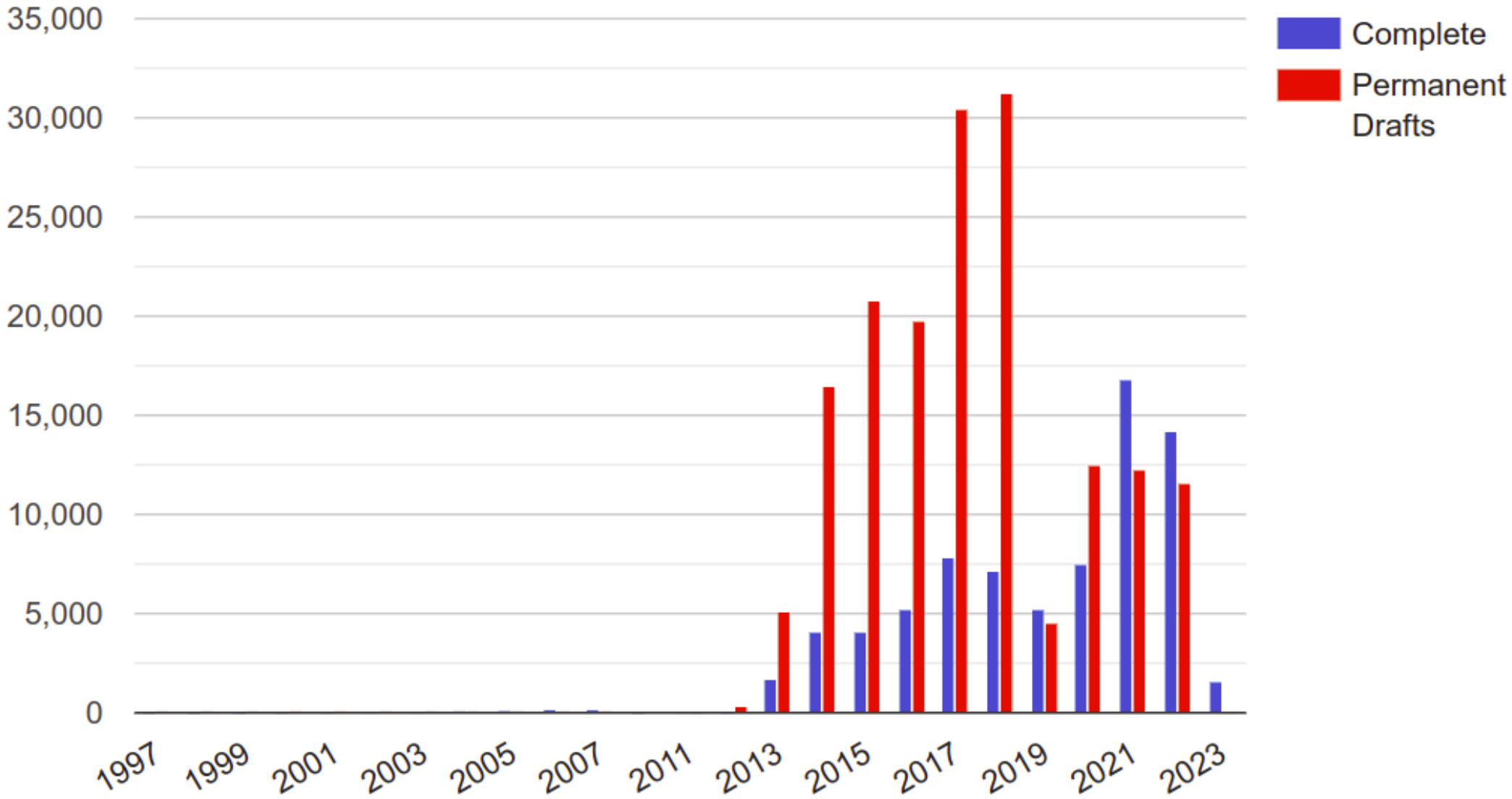
Nature, 464:670-671, 2010.



Sequenced Genomes

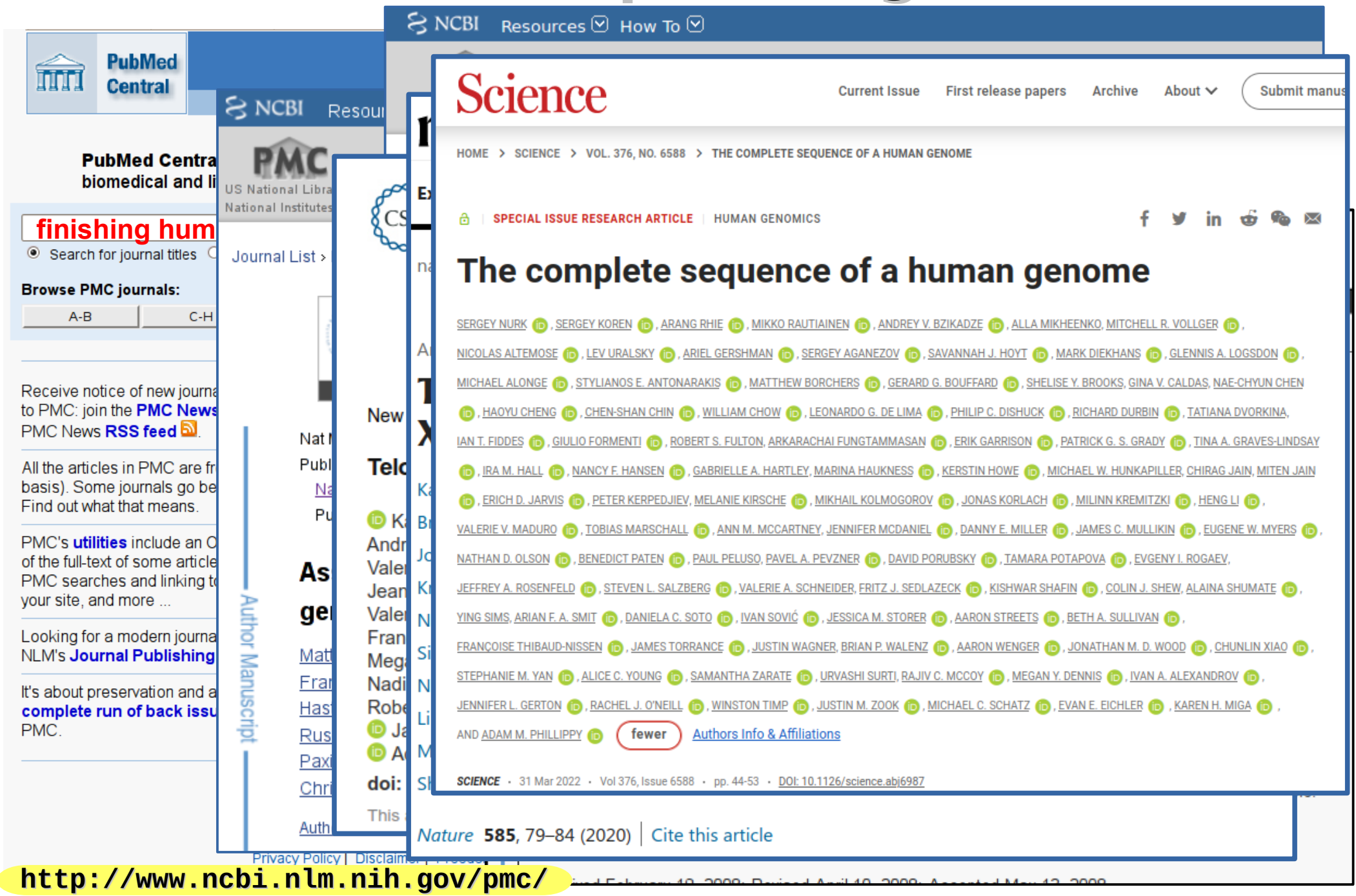


Genome Completeness



https://gold.jgi.doe.gov/statistics

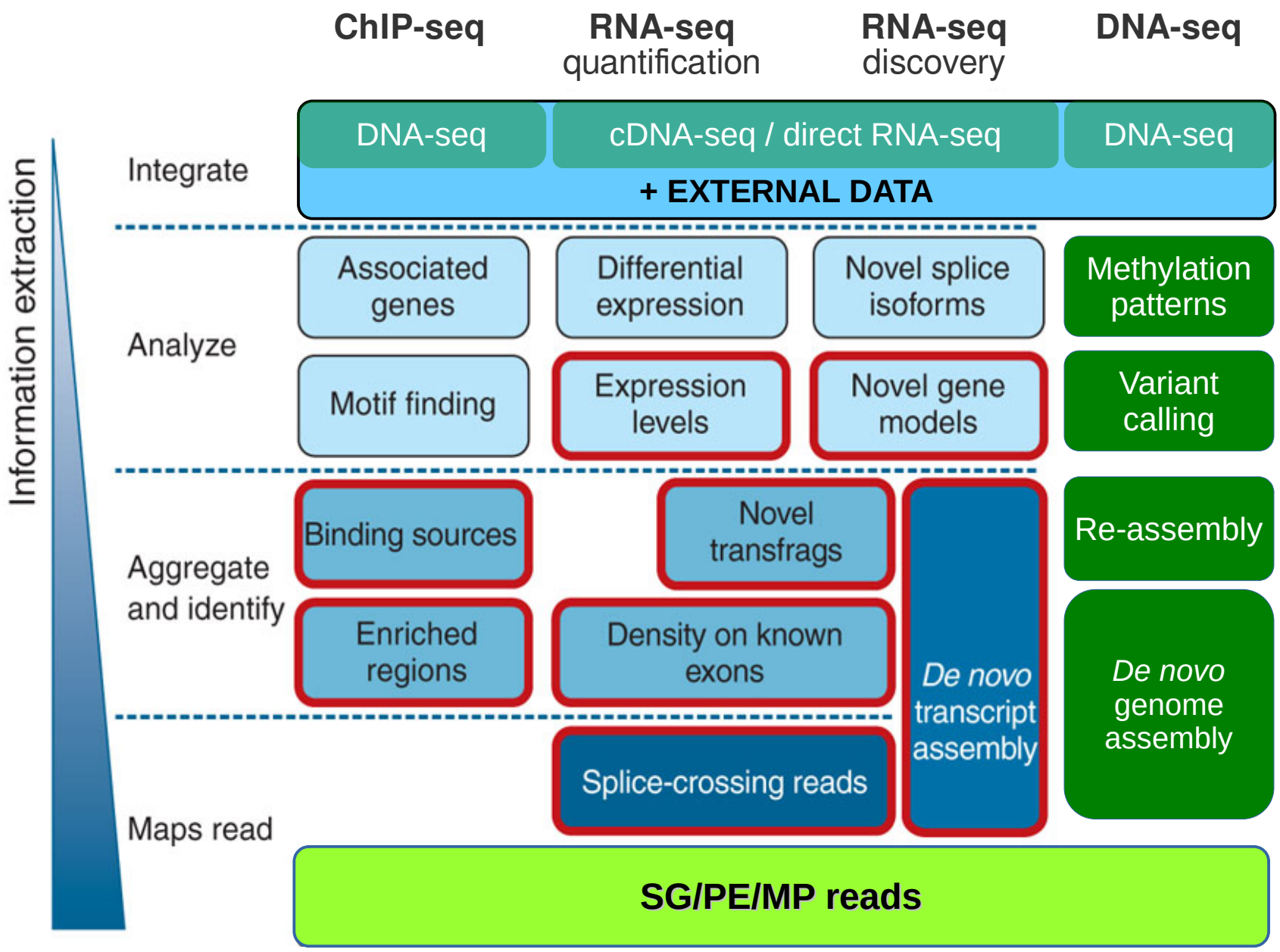
How much complete a genome is?



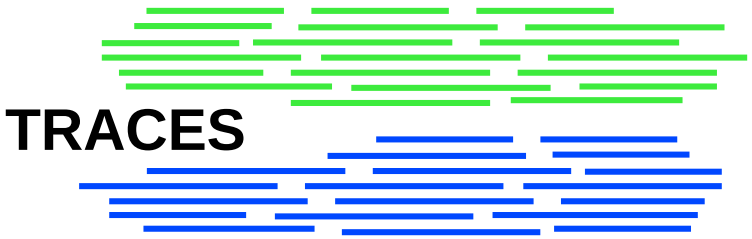
http://www.ncbi.nlm.nih.gov/pmc/

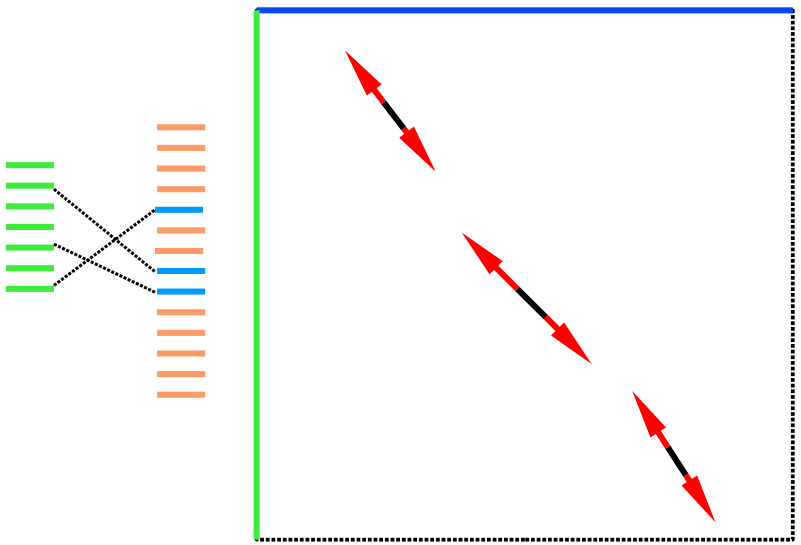
SEQUENCING OVERVIEW

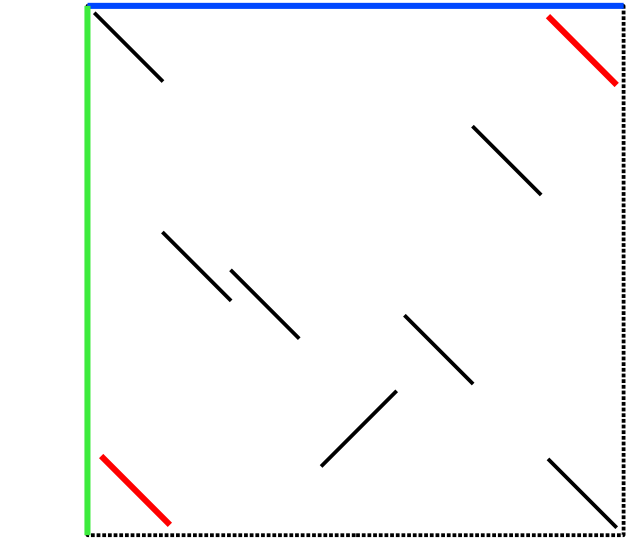
Seqs, Seqs & more Seqs

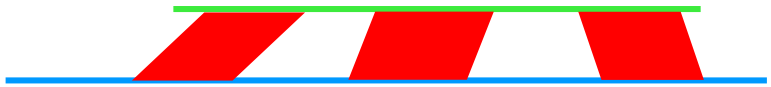


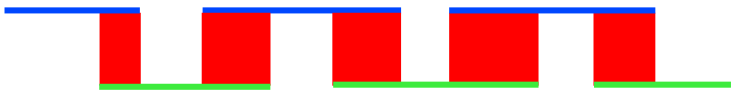
Assembly vs Homology



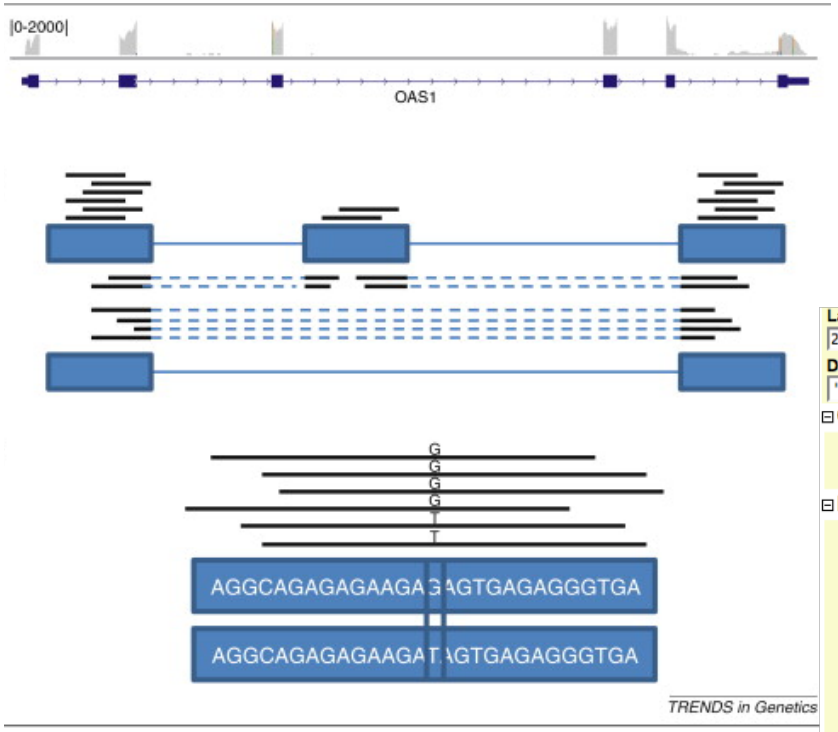


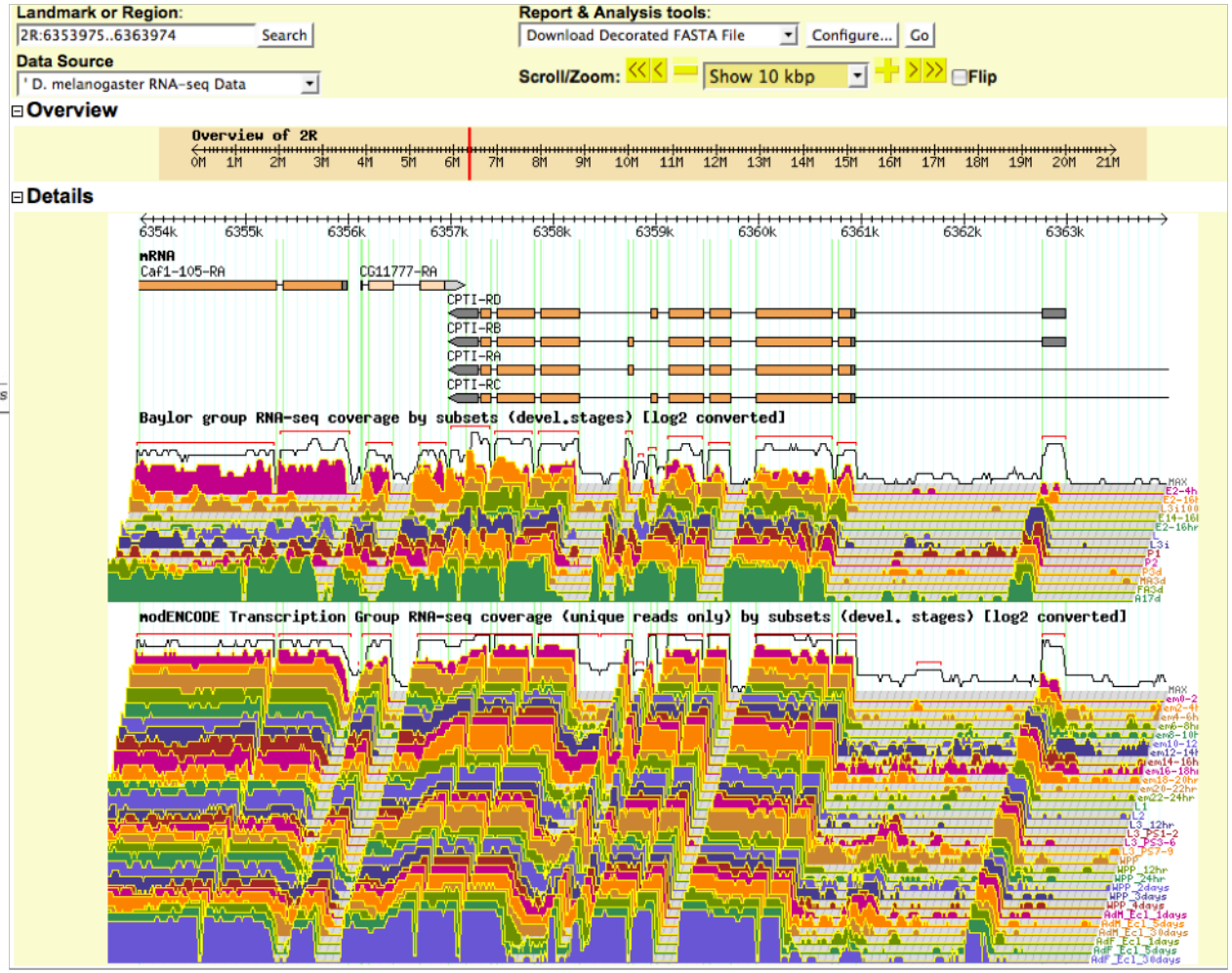




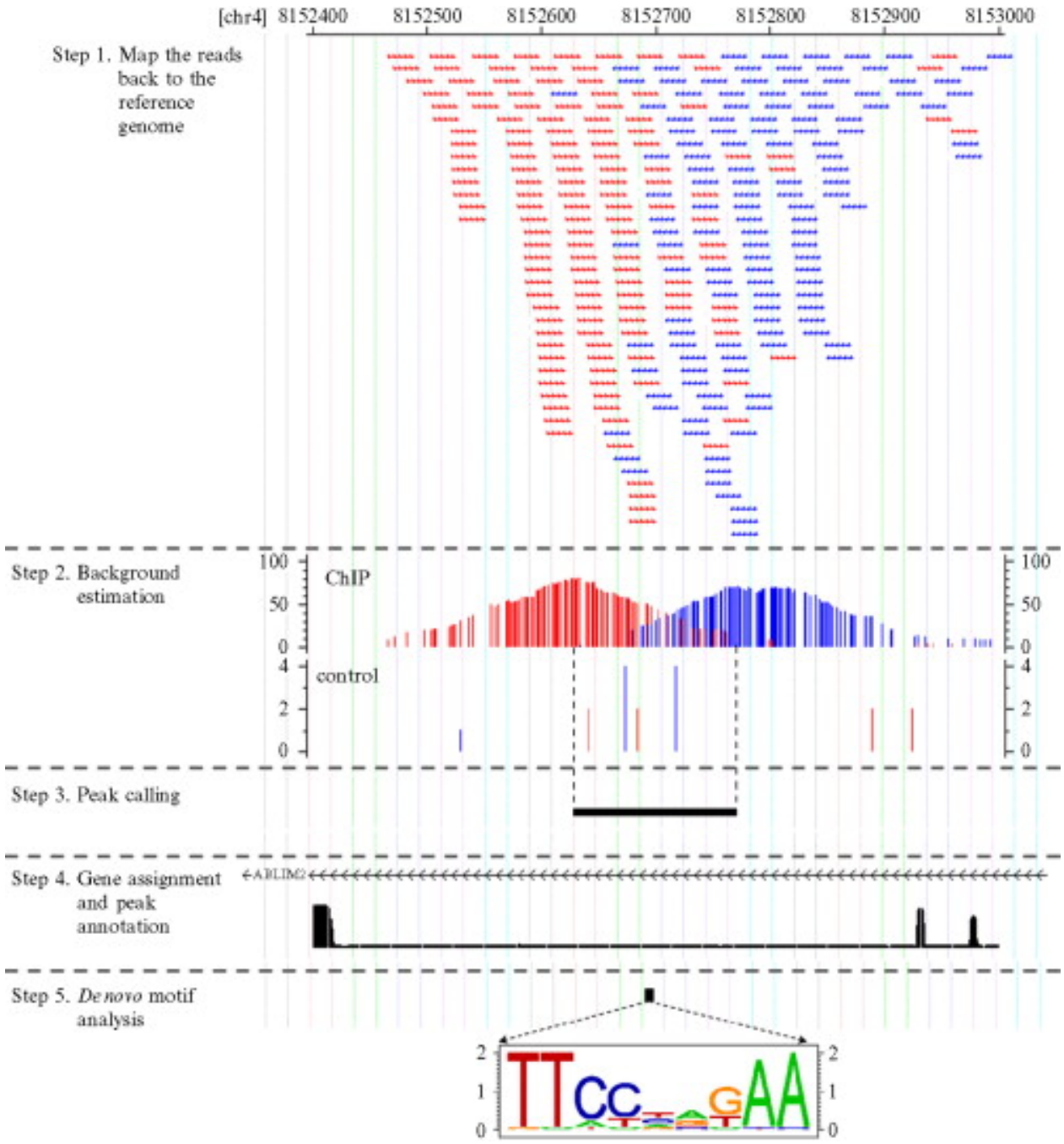


RNA-Seq Overview

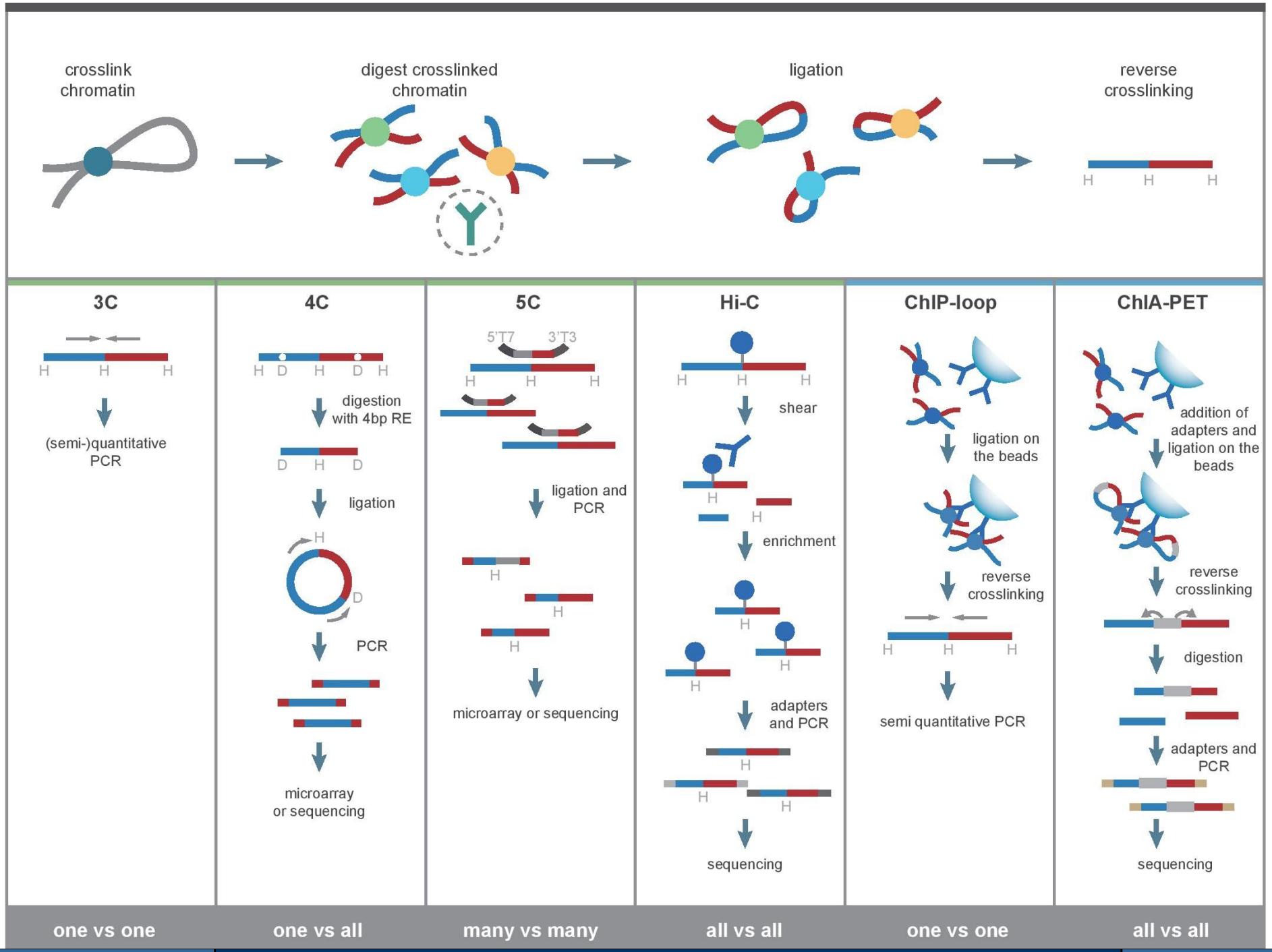




From Reads to Peaks to Signals

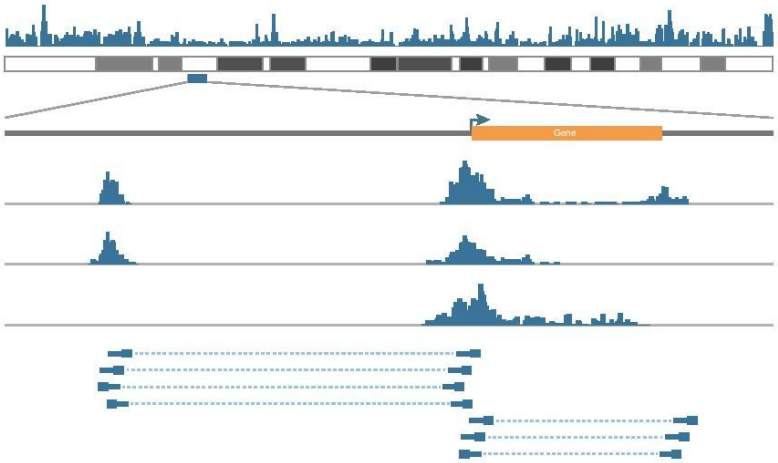


Chromatin Conformation Overview

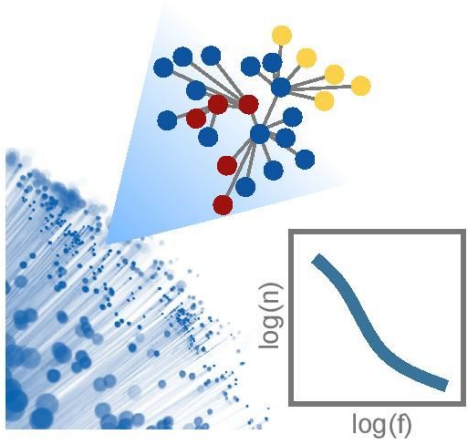


Li et al *BMC Genomics* 2014

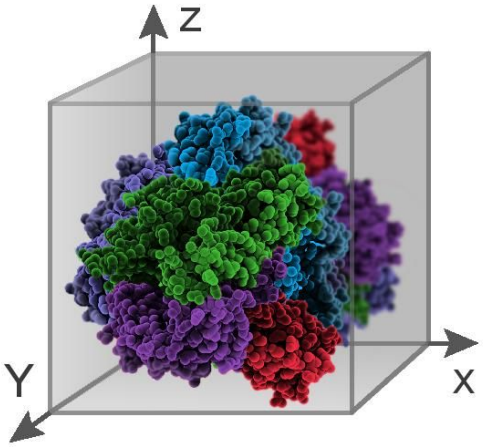
Chromatin Conformation Over Genome



1D
Linear map

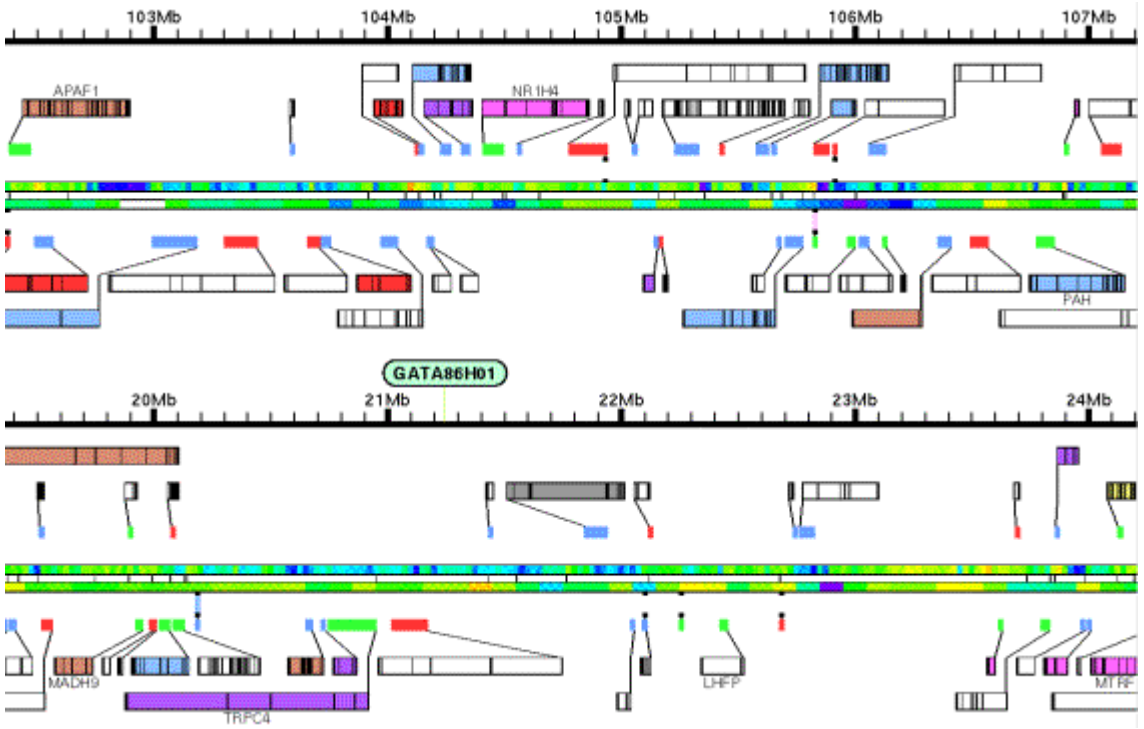


2D
Network(scale-free)

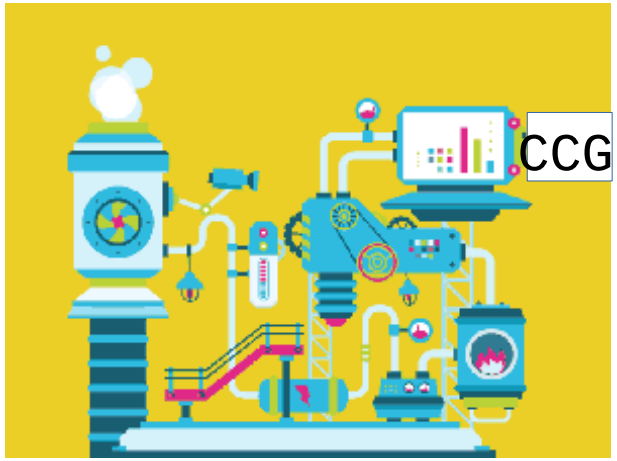


3D
Structure&dynamics

SEQUENCE ANNOTATION



Annotating Sequences: Does it matters ?

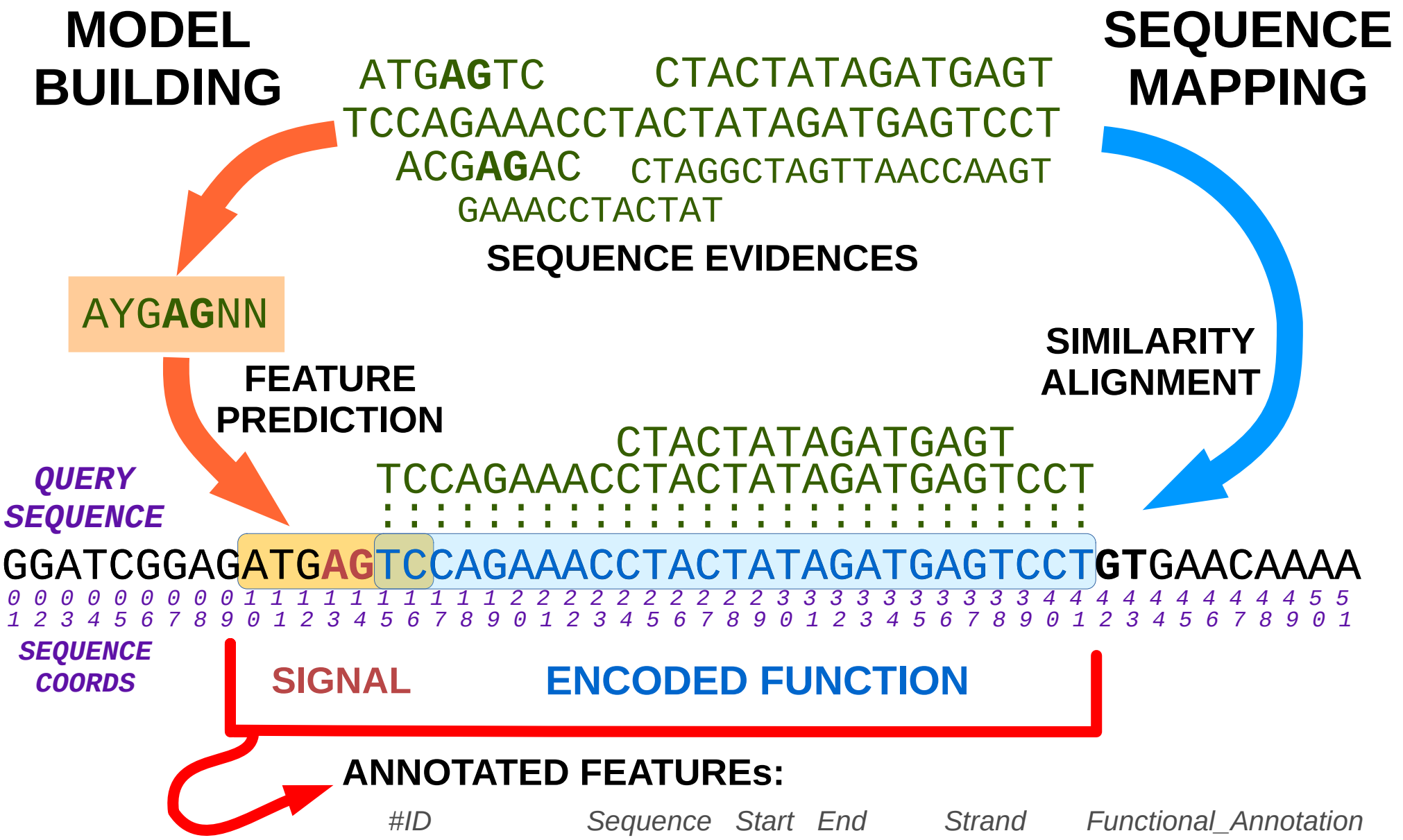


"The genome sequence of an organism is an information resource unlike any that biologists have previously had access to.

But the **value** of the **genome** is only as good as its **annotation**. It is the annotation that bridges the gap from the sequence to the biology of the organism."

Stein L. *Nat Rev Genet*, 2(7):493-503, 2001

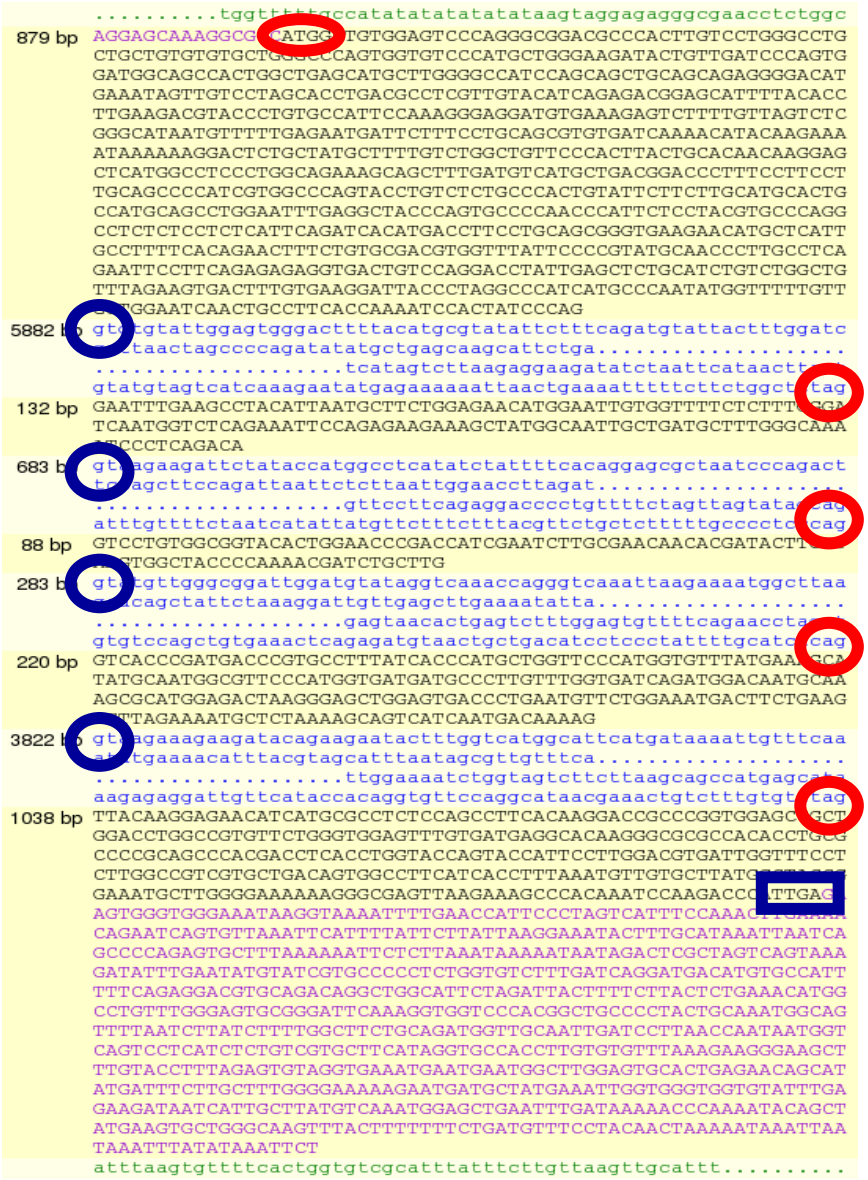
Sequence Annotation: What Does It Mean?



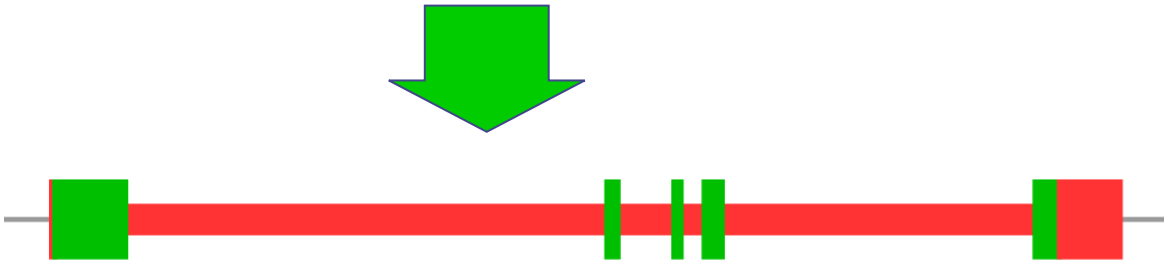
ANNOTATED FEATUREs:

#ID	Sequence	Start	End	Strand	Functional_Annotation
SPLICESITE	QUERY	10	16	FORWARD	U2-GTAG
EXON	QUERY	15	41	FORWARD	KINASE_DOMAIN

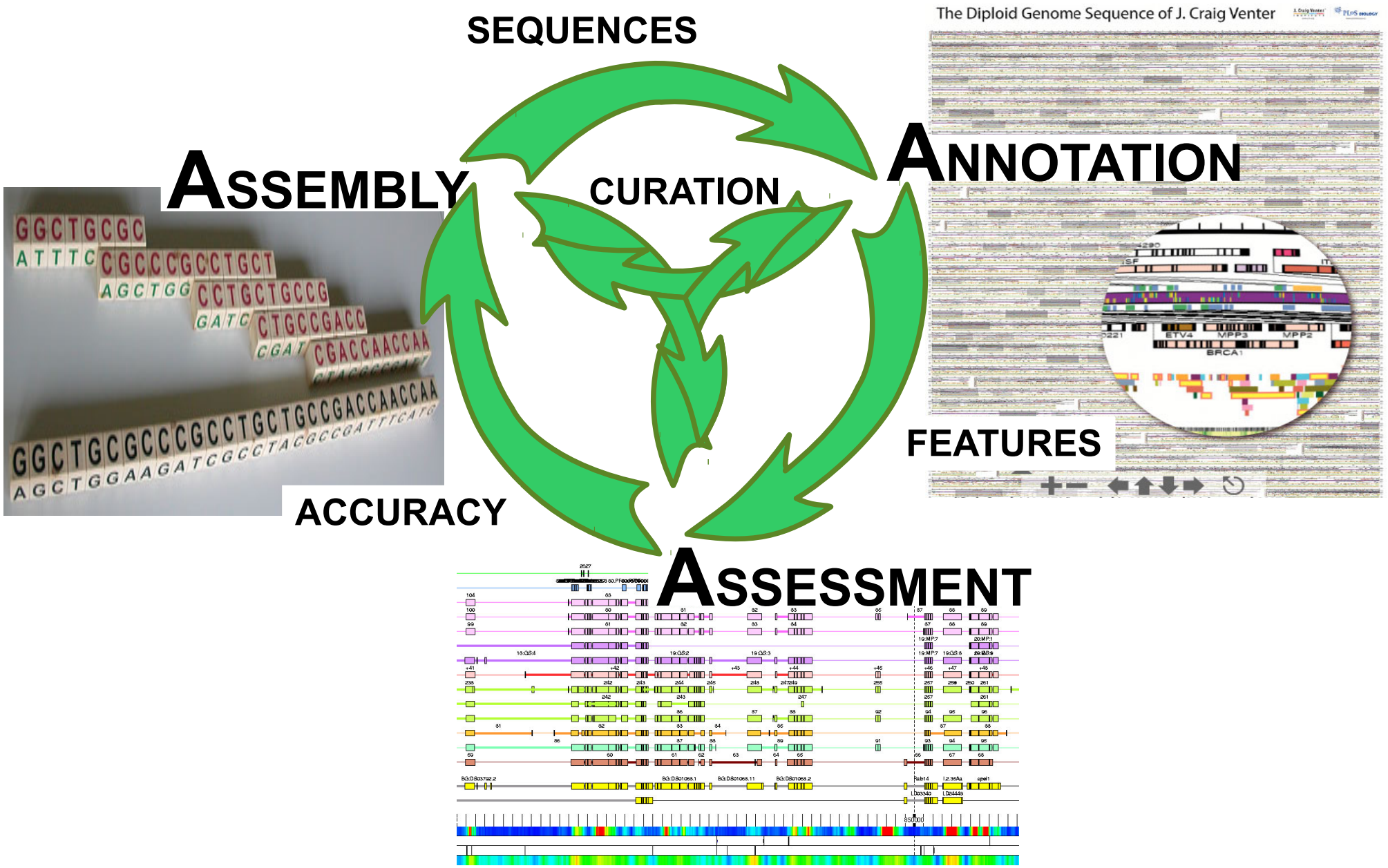
ANNOTATING SEQUENCES



```
chr2  refseq  mRNA          1  13027  .  +  .  transcript_id "UGT1A1"
chr2  refseq  exon          1    879  .  +  .  transcript_id "UGT1A1"
chr2  refseq  start_codon  16     18  .  +  .  transcript_id "UGT1A1"
chr2  refseq  CDS          16    879  .  +  0  transcript_id "UGT1A1"
chr2  refseq  exon       6762   6893  .  +  .  transcript_id "UGT1A1"
chr2  refseq  CDS        6762   6893  .  +  1  transcript_id "UGT1A1"
chr2  refseq  exon       7577   7664  .  +  .  transcript_id "UGT1A1"
chr2  refseq  CDS        7577   7664  .  +  1  transcript_id "UGT1A1"
chr2  refseq  exon       7948   8167  .  +  .  transcript_id "UGT1A1"
chr2  refseq  CDS        7948   8167  .  +  2  transcript_id "UGT1A1"
chr2  refseq  exon      11990  13027  .  +  .  transcript_id "UGT1A1"
chr2  refseq  CDS       11990  12287  .  +  0  transcript_id "UGT1A1"
chr2  refseq  stop_codon  12285  12287  .  +  .  transcript_id "UGT1A1"
```



The Annotation Problem



Assembly vs Annotation VERSIONS

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Search Human...

Human (GRCh37) ▼

Human assembly and gene annotation

Assembly

This site provides a data set based on the February 2009 *Homo sapiens* high coverage assembly GRCh37 from the Genome Reference Consortium. This assembly is used by UCSC to create their hg19 database. The data set consists of gene models built from the genewise alignments of the human proteome as well as from alignments of human cDNAs using the cDNA2genome model of exonerate.

This release of the assembly has the following properties:

- 27478 contigs.
- contig length total 3.2 Gb.
- chromosome length total 3.1 Gb.

It also includes nine haplotypic regions, mainly in the MHC region of chromosome 6.

Watch a video on YouTube about patches and haplotypes in the Human genome.

Gene annotation

The Ensembl human gene annotations have been updated using Ensembl's a pipeline. The updated annotation incorporates new protein and cDNA sequenc publicly available since the last GRCh37 genebuild (March 2009).

In release 70 (January 2013), we continue to display a joint gene set based or automatic annotation from Ensembl and the manually curated annotation from set corresponds to GENCODE release 15. The Consensus Coding Sequence been mapped to the annotations. More information about the CCDS project.

Updated manual annotation from Havana is merged into the Ensembl annotat Transcripts from the two annotation sources are merged if they share the sam boundaries (i.e. have identical splicing pattern) with slight differences in the te Importantly, all Havana transcripts are included in the final Ensembl/Havana n set. In this release, 23532 Ensembl gene models and 49095 Havana genes w create the final set of 60620 genes.

- Detailed information on genebuild (PDF)

Statistics

Summary

Gene counts (Primary assembly)

Coding genes:	20,848
Non coding genes:	22,486
Pseudogenes:	13,430
Gene transcripts:	195,409

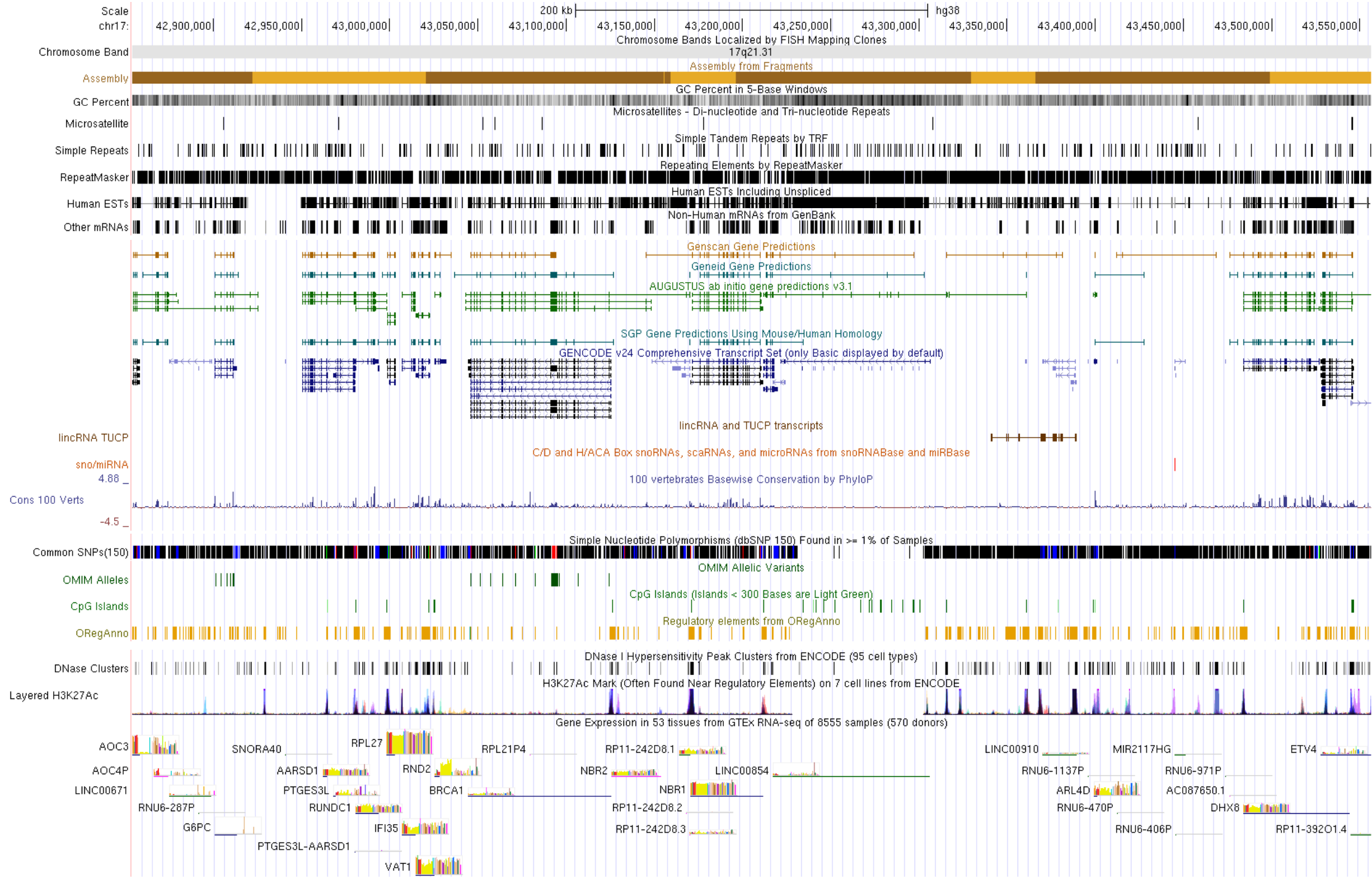
Gene counts (Alternate sequences)

Coding genes:	2,442
Non coding genes:	1,325
Pseudogenes:	1,538
Gene transcripts:	17,863

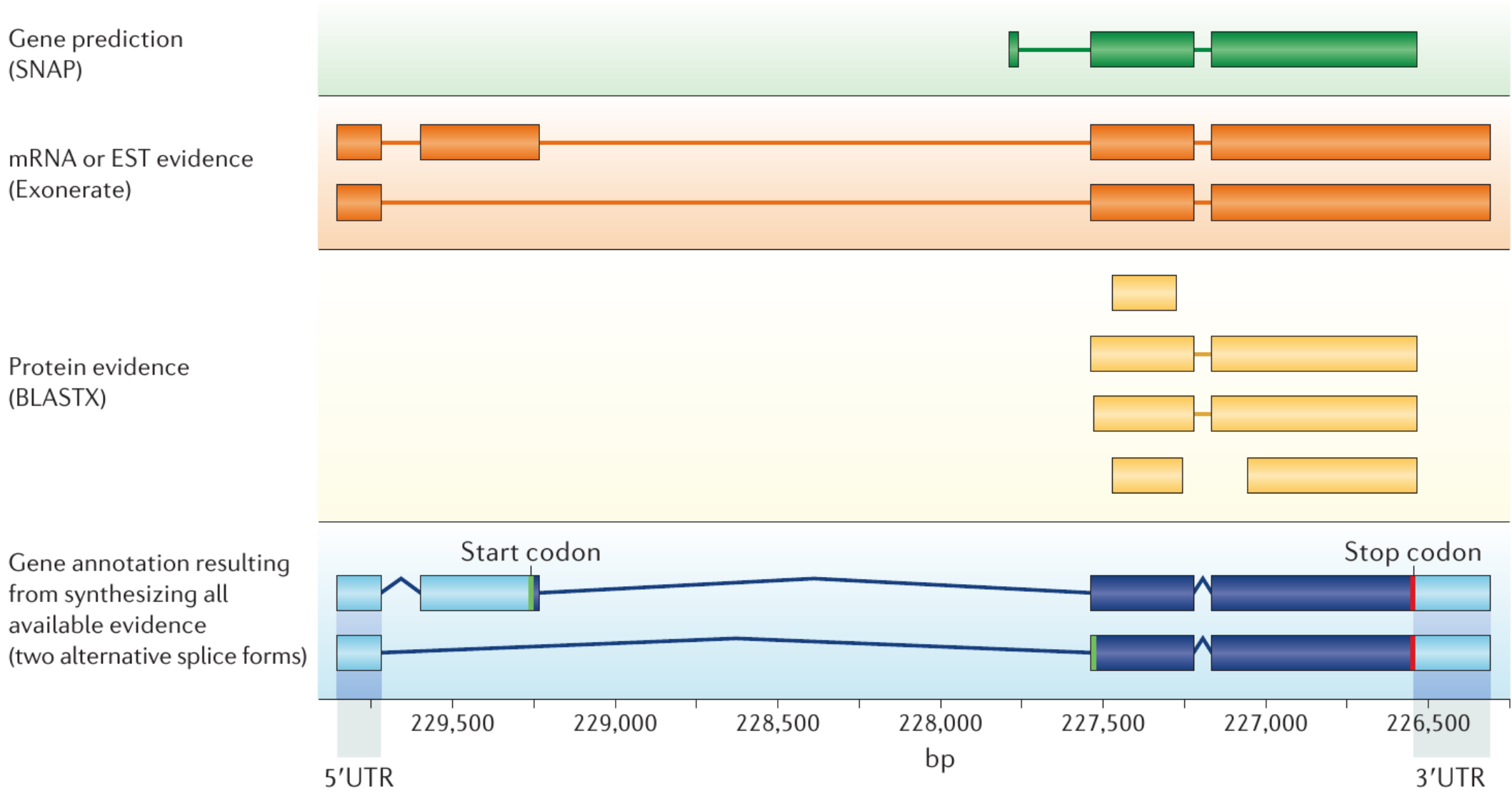
Other

Genscan gene predictions:	48,186
Short Variants (SNPs, indels, somatic mutations):	54,474,297
Structural variants:	10,152,873

Annotation Layers

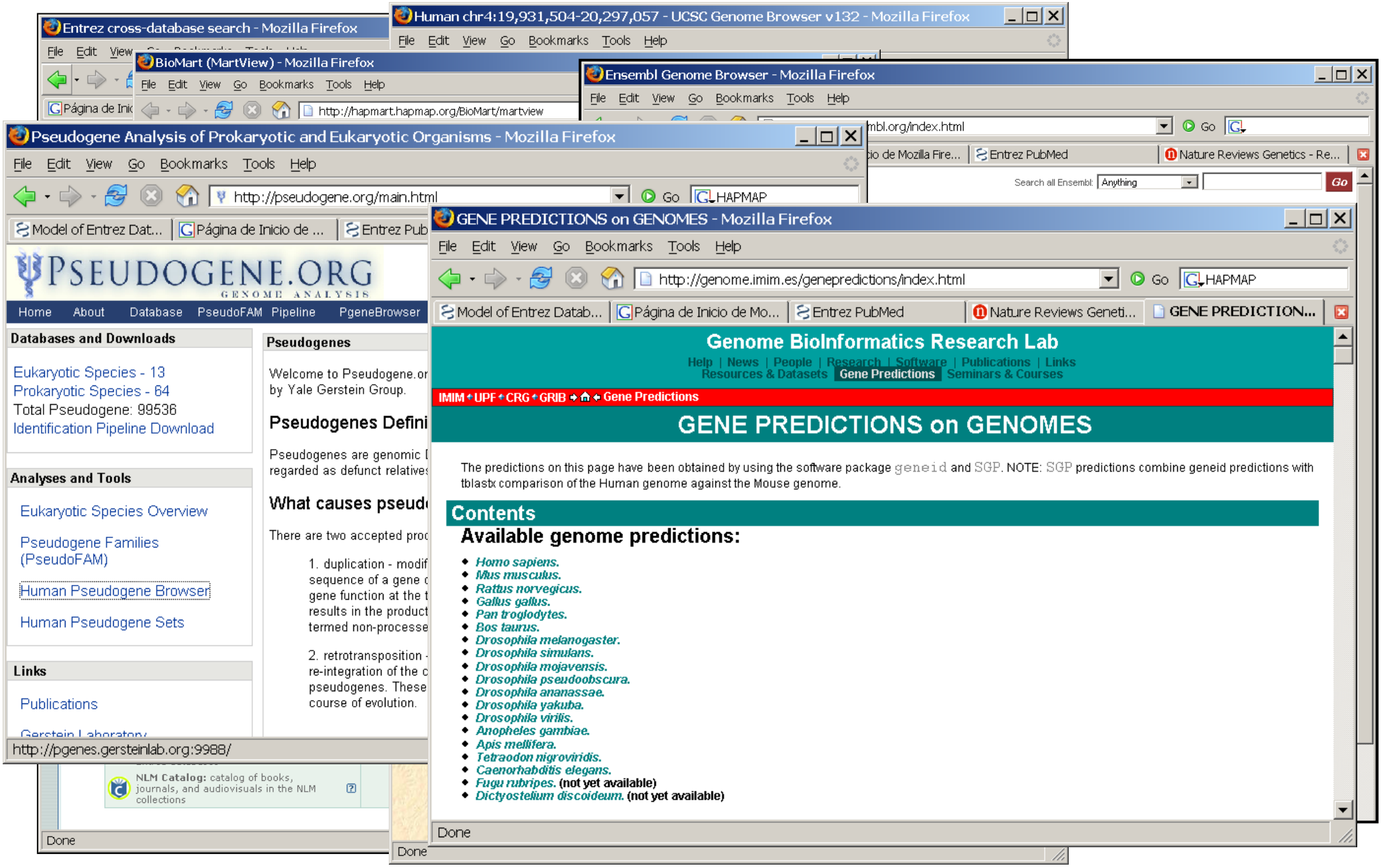


Gene-Finding vs Gene Annotation



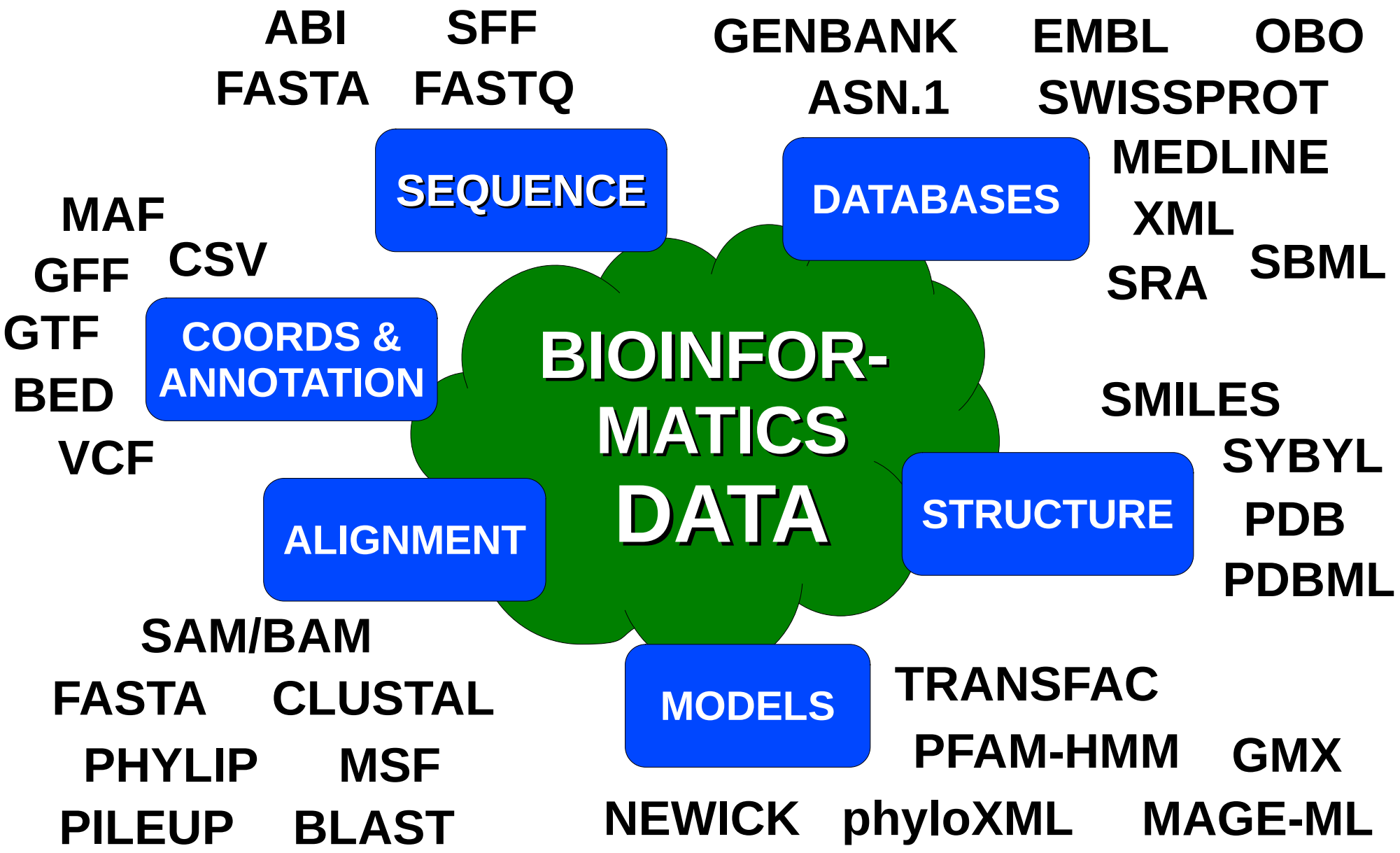
Yandell & Ence, *Nat Rev Genet*, 13:329, 2012.

Surfing a Web of Annotations



DATA FORMATS

A Plethora of Bioinformatics Formats



TABULAR vs MULTILINE SEQUENCE RECORDS

```
#SEQUENCE_ID    NUCLEOTIDE_SEQUENCE                    EXTRA_FIELDS
dna_seq_1   ACCCGGGTCTTGACCG...GACCATCTCCGAAAT   description_1
dna_seq_2   AAGATCCCAACATAGG...ACTAC   description_2
```

FIELD SEPARATOR " " or "\t"

RECORD SEPARATOR ">"

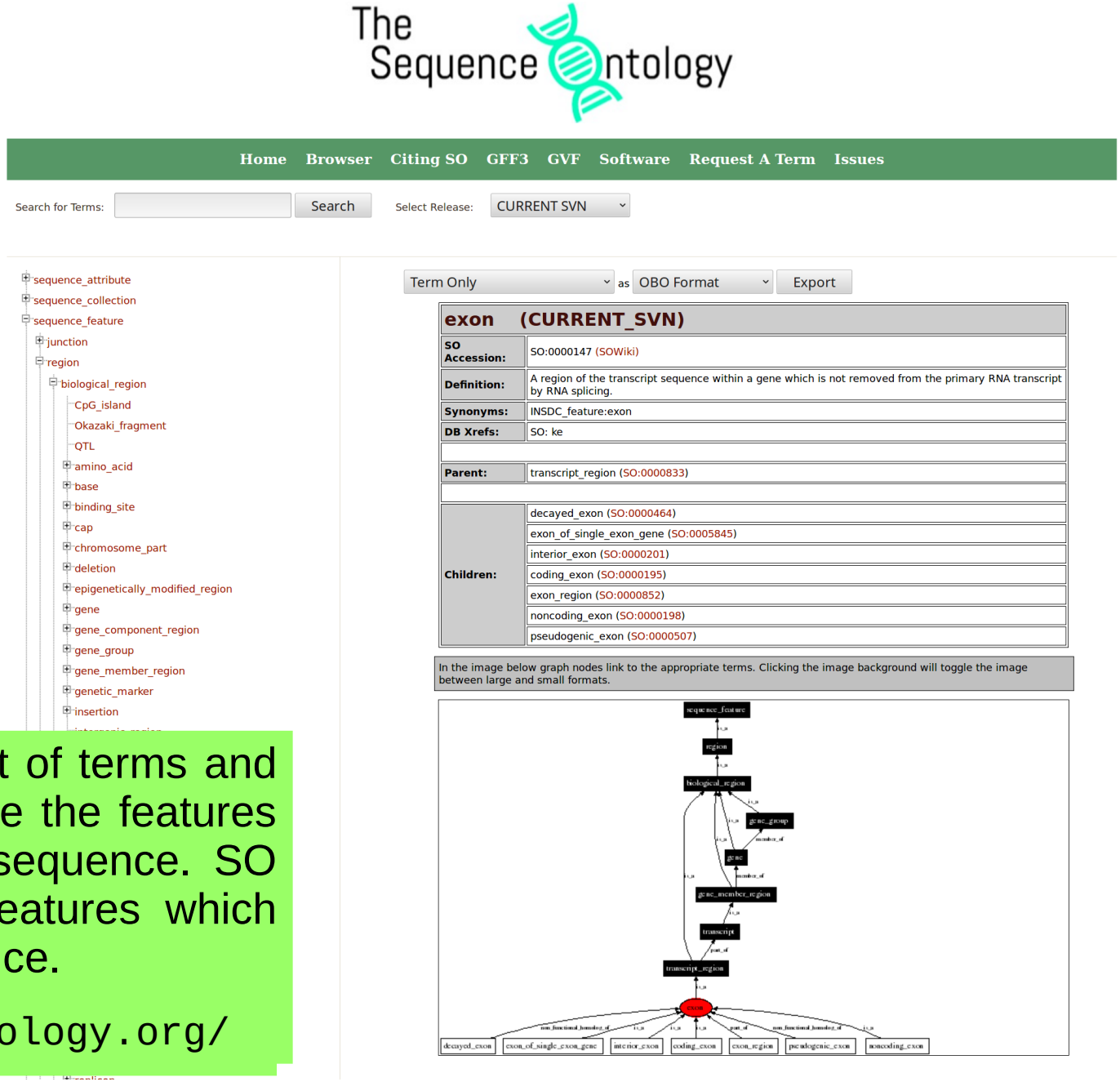
RECORD SEPARATOR "\n"

```
>dna_seq_1 description_1
ACCCGGGTCTCATCCCTTGACCCTG
GGAACTCGCCTTCTCTGCCTTTCCC
...
GCCTCCCCTCTTGTTCTGGGGGGGG
GGAACTCGCCTTCTCTGCCTTTCCC
GACCATCTCCGAAAT
>dna_seq_2 description_2
AAGATCCCAAATGTGAACACTCATA
GGTGACTTCCAGTTCCGCCCAGTGA
...
CGTAGGAAGTCCAGCTGAGGCGCCT
ACTAC
```

Sequence Ontology (SO)

DEFINE and DESCRIBE DIFERENCES AMONG ANNOTATION FEATURES

GENOMIC
cDNA
EST
GENE
primary TRANSCRIPT
mRNA
EXON
UTR
CDS
ORF
...



Sequence Ontology is a set of terms and relationships used to describe the features and attributes of biological sequence. SO includes different kinds of features which can be located on the sequence.

`http://www.sequenceontology.org/`

STRANDNESS

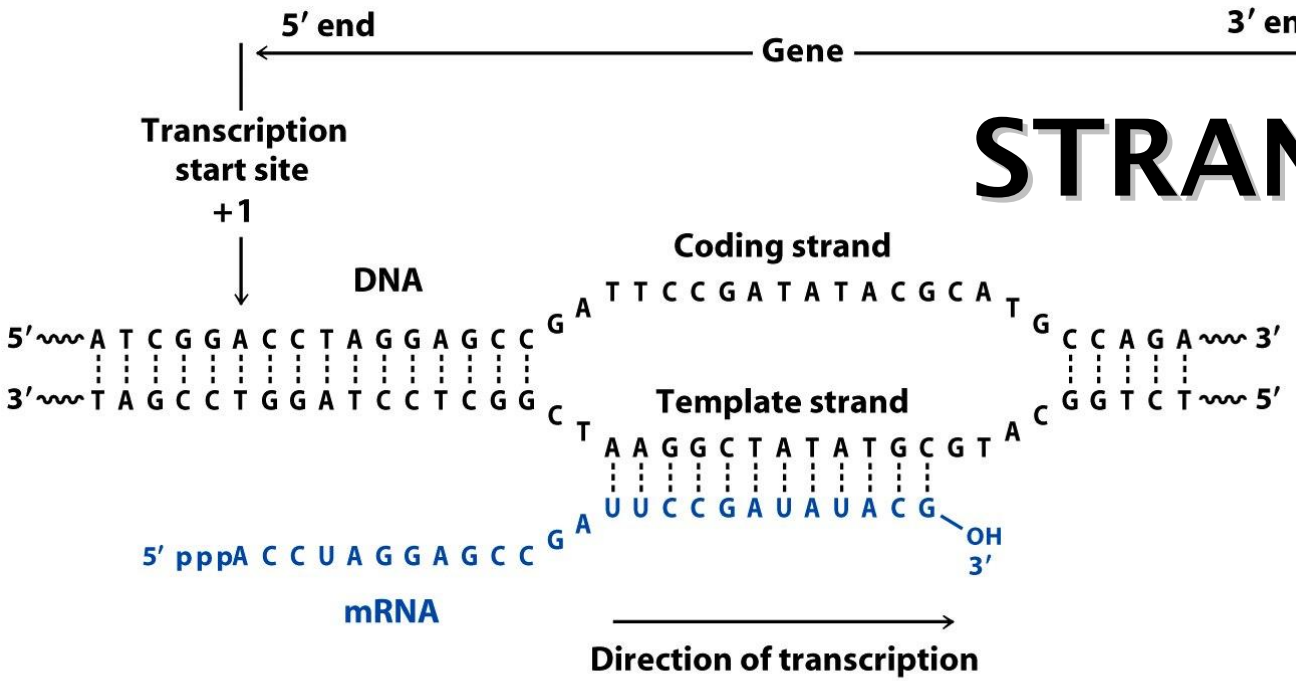


Figure 21-5 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

SEQUENCE

ANNOTATIONS

Forward [5’→3’]

TGTGAATACAAGGCAGAACCTAGACCATGTTAAAGAATCAAAAACAGGTT

FORWARD [+]

Complement

BASE PAIR COMPLEMENTARITY

ACACTTATGTTCCGTCTTGGATCTGGTACAATTTCTTAGTTTTTGTCCAA

Reverse

TTGGACAAAAACTAAGAAATTGTACCAGATCCAAGACGGAACATAAGTGT

Reverse-Complement

BASE PAIR COMPLEMENTARITY

REVERSE [-]

[Forward *3’→5’*] AACCTGTTTTTGATTCTTTAACATGGTCTAGGTTCTGCCTTGTATTCACA

ANNOTATION FORMATS

RAW COORDS

```
>NM_005101 chr1
88946 89980 mrna
88946 89020 utr
89021 89023 cds
89431 89925 cds
89926 89980 utr
```

```
<NM_021170 chr1
74412 75537 mrna
74412 74505 utr
74506 74879 cds
74973 75060 cds
75313 75420 cds
75421 75537 utr
```

GENERAL FEATURE FORMAT (GFF)

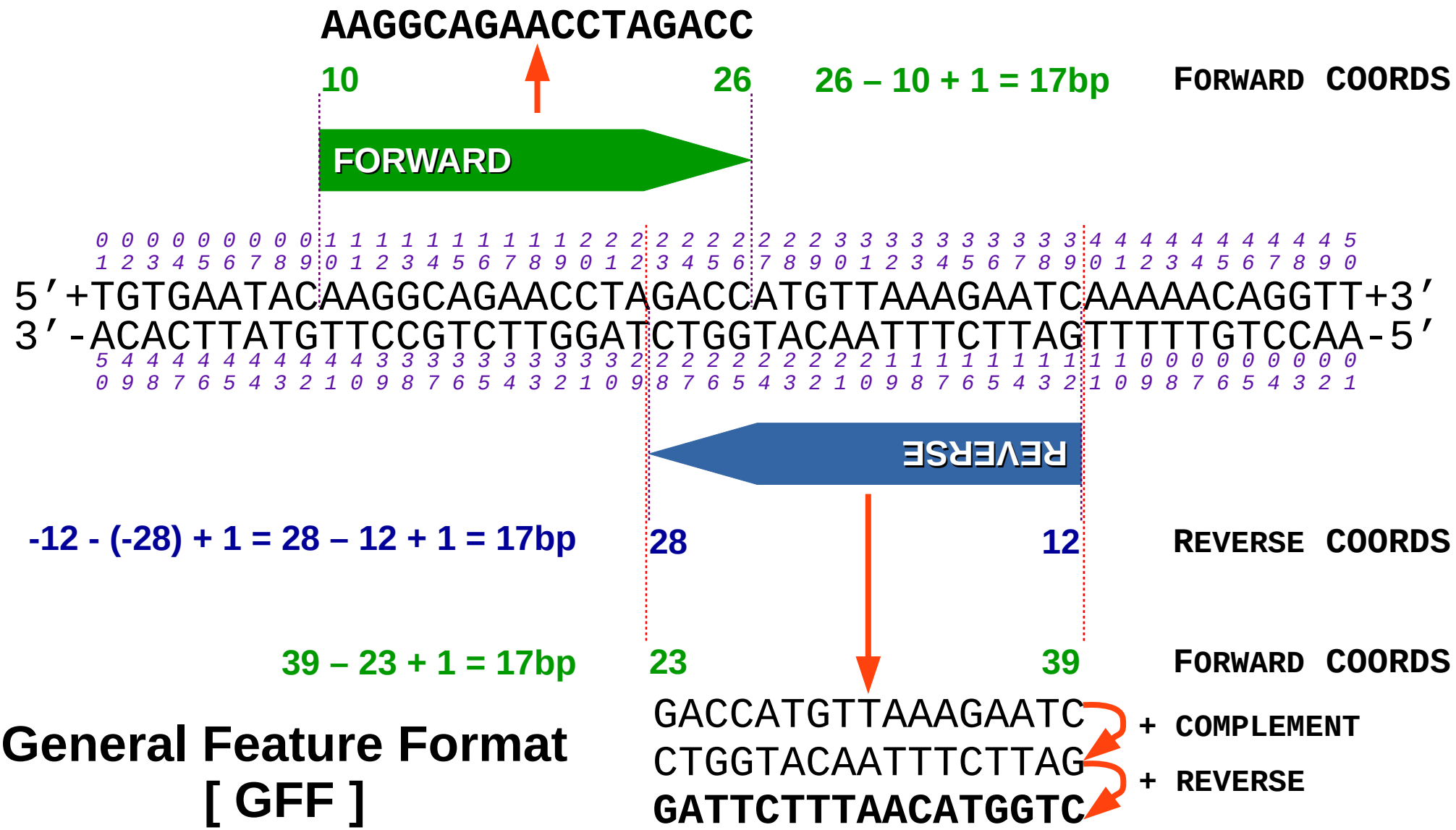
```
# Seq: chr1 Gene: NM_021170
chr1 refseq mrna 74412 75537 . - . NM021170
chr1 refseq utr  74412 74505 . - . NM021170
chr1 refseq cds  74506 74879 . - 2 NM021170
chr1 refseq cds  74973 75060 . - 0 NM021170
chr1 refseq cds  75139 75234 . - 0 NM021170
chr1 refseq cds  75313 75420 . - 0 NM021170
chr1 refseq utr  75421 75537 . - . NM021170
```

UCSC BED

```
# GENE SEQUENCE STRAND mRNA_START mRNA_STOP CDS_START CDS_STOP EXONS EXONS_START EXONS_END

NM_024796   chr1   -   801451   802749   801942   802434   1   801451, 802749,
NM_021170   chr1   -    74411    75537    74505    75420   4 \
                      74411,74972,75138,75312,   74879,75060,75234,75537,
...
```

Strands & Annotation Coords

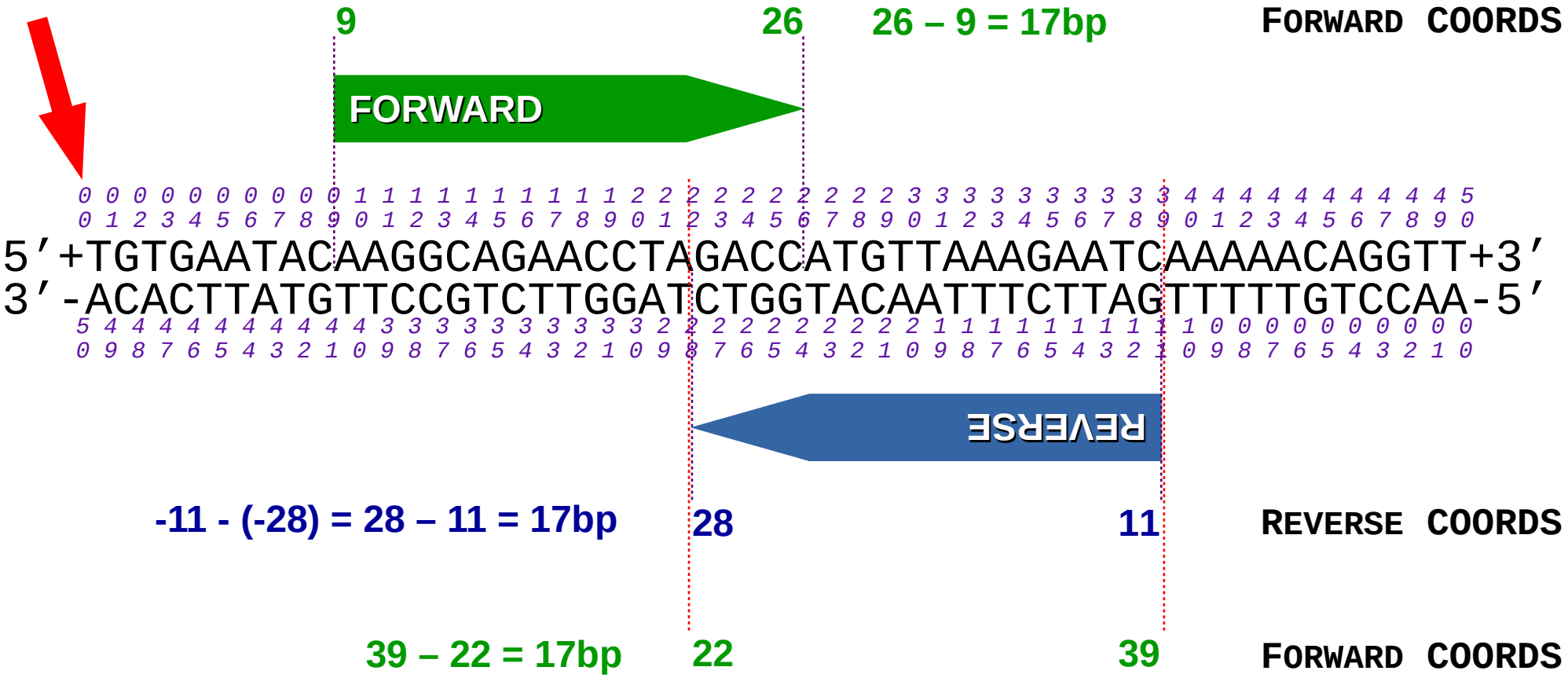
FULLY-CLOSED INTERVALS / 1-BASED



General Feature Format
[GFF]

Strands & Annotation Coords

HALF-OPEN INTERVALS / 0-BASED



UCSC BED Format

More examples at http://genome.ucsc.edu/blog/the-ucsc-genome-browser-coordinate-counting-systems/

COMPLEX RECORDS: GENBANK

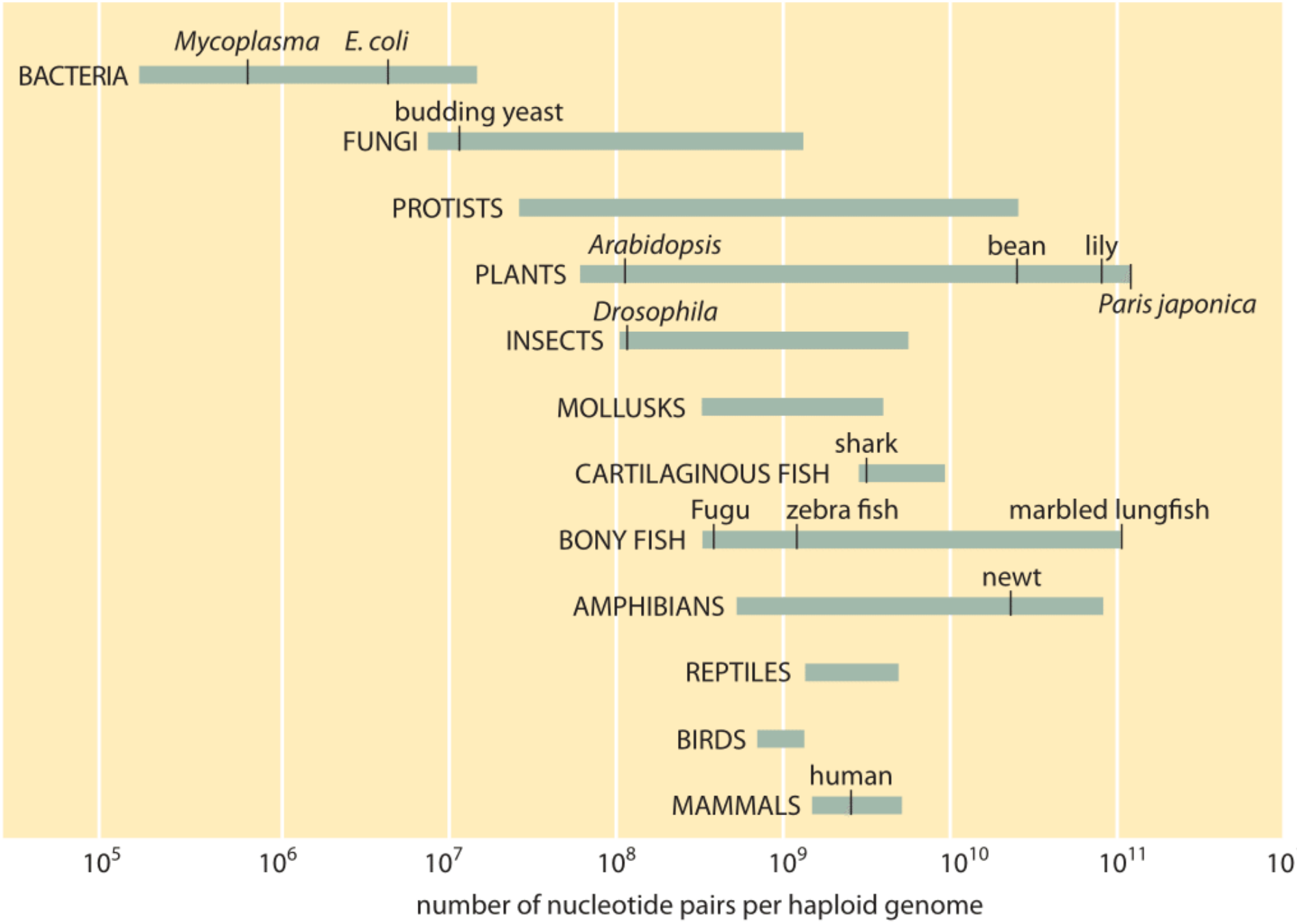
```
LOCUS       NM_021170 893 bp mRNA linear PRI 24-SEP-2005
DEFINITION  Homo sapiens hairy and enhancer
            of split 4 (Drosophila)(HES4), mRNA.
ACCESSION   NM_021170
VERSION     NM_021170.2 GI:20127596
...
SOURCE      Homo sapiens (human)
FEATURES    Location/Qualifiers
  source    1..893
            /organism="Homo sapiens"
            /mol_type="mRNA"
            /db_xref="taxon:9606"
            /chromosome="1"
            /map="1p36.33"
  gene      1..893
            /gene="HES4"
            /db_xref="GeneID:57801"

  ...

  CDS       118..783
            /gene="HES4"
            /note="bHLH factor Hes4"
            /codon_start=1
            /product="hairy and enhancer of split 4"
            /protein_id="NP_066993.1"
            /db_xref="GI:10863967"
            ...
            /translation="MAADTPGKPSASPMAGAPASASRTPDKP
                          ...
                        LLPGLTRALPAAPRAGPQGPGGPWRPWLR"

  ...

ORIGIN
        1 gggaaagaat gcggagccgg gttcacacac cccgcggcgg cgaggcctta aatagggaaa
       61 cggcctgagg cgcgcgcggg cctggagccg ggatccgccc taggggctcg gatcgccgcg

          ...

      901 ccgttctagg gccgtggcct ttgccgagac tgtagcagag aaaacgtatt tattattcca
      961 ga
//
```

SEQUENCE STATISTICS

Basic Genome Stats

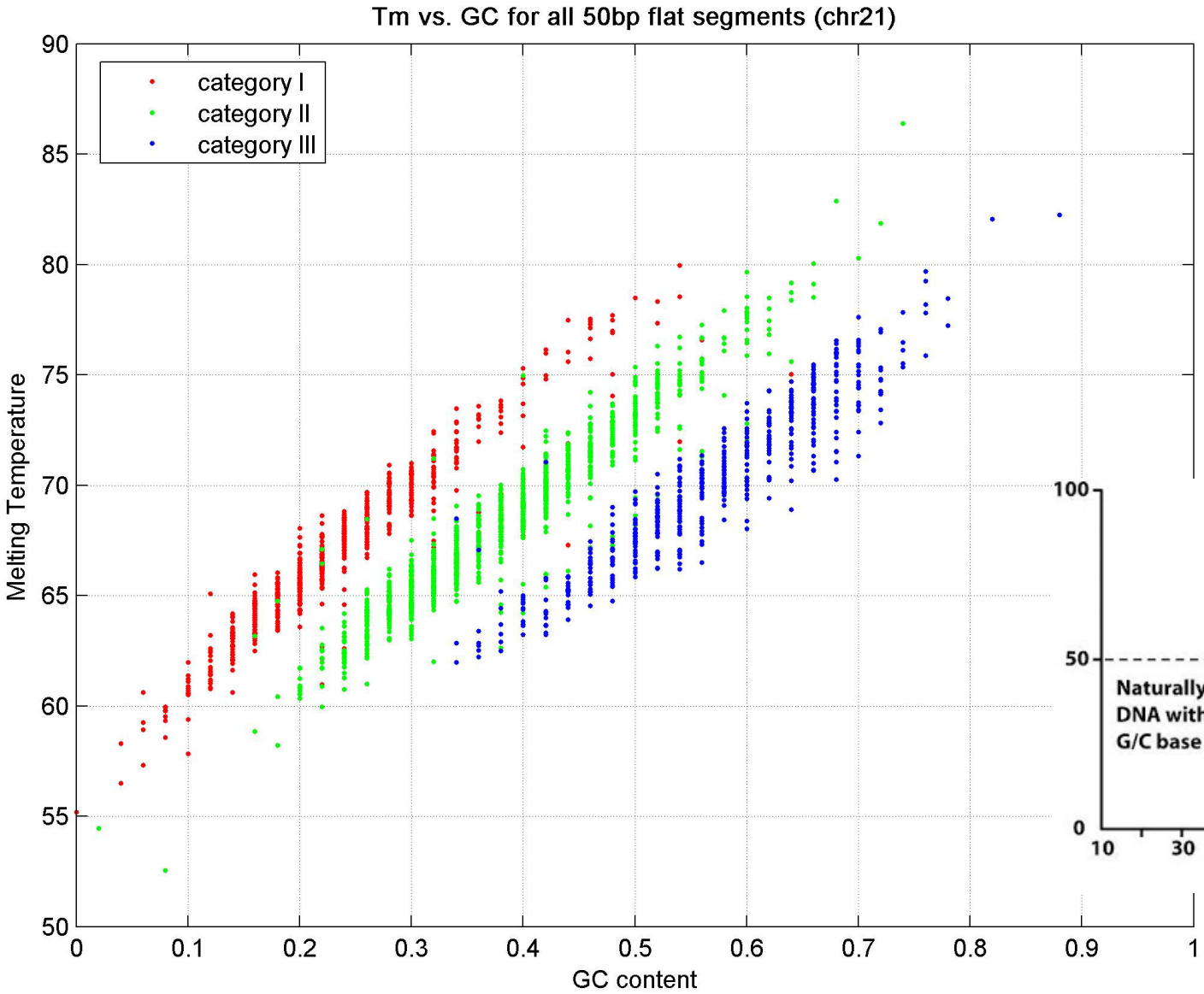
SPECIES	LENGTH (*bp*)	#GENES	GC Content
HIV Virus **(ssRNA)**	9,780	9	42.5
Lambda bacteriophage ***(dsDNA)***	49,500	73	49.9
Mycoplasma genitalium	580,000	476	31.7
Haemophilus influenzae	1,800,000	2,098	37.9
Escherichia coli	4,609,000	5,449	50.6
Saccharomyces cerevisiae	14,625,900	6,692	40.7
Plasmodium falciparum	23,326,900	5,362	19.3
Caenorhabditis elegans	98,302,800	20,222	35.4
Arabidopsis thaliana	118,891,000	27,655	38.9
Drosophila melanogaster	143,726,000	13,931	42.1
Takifugu rubripes	391,485,000	18,523	45.8
Homo sapiens	3,257,320,000	20,376	41.5
Mus musculus	3,251,250,000	22,628	41.8
Triticum aestivum	15,344,700,000	110,790	46.1

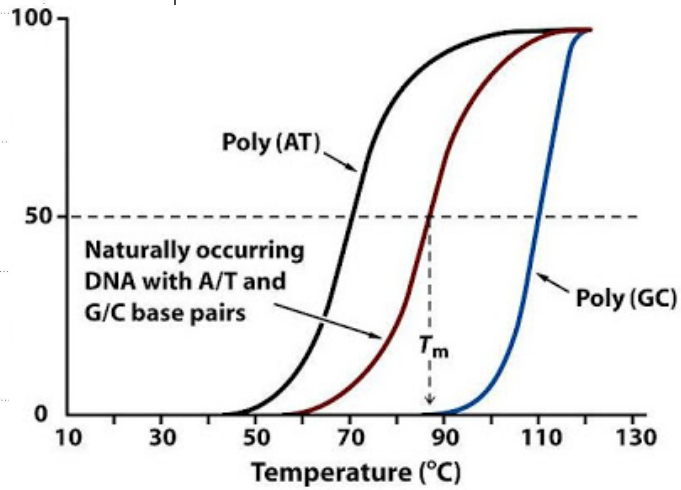
C-VALUE Paradox



Thomas, C.A.Jr. *Annu. Rev. Genet.* 1971

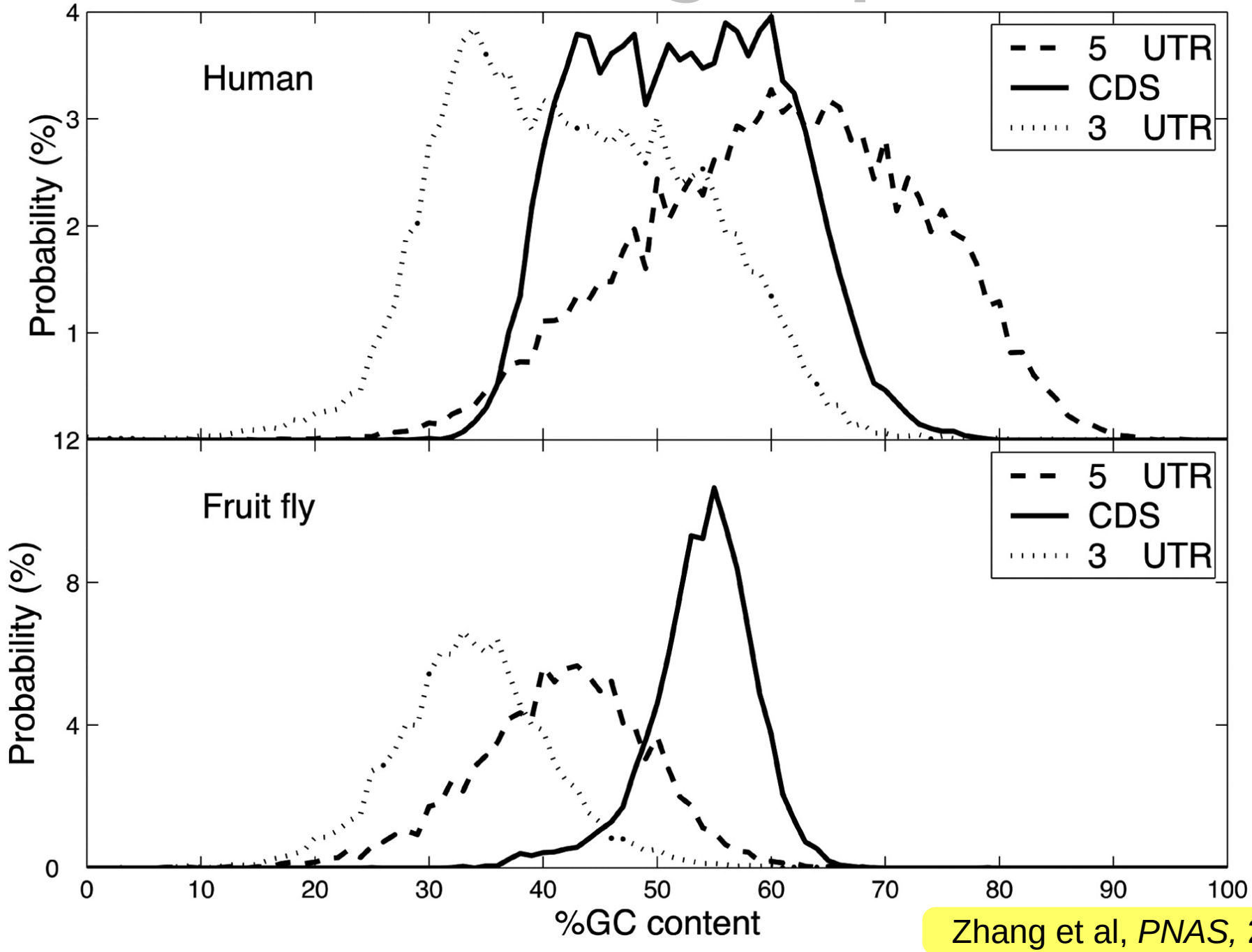
GC Content Affects Tm





Liu et al. *PloS Comp Biol* 2007

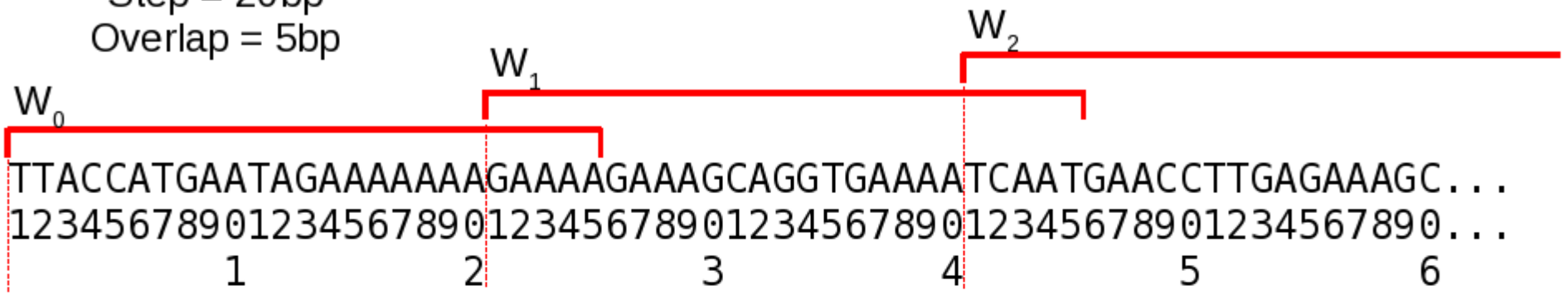
%GC on Coding Sequences



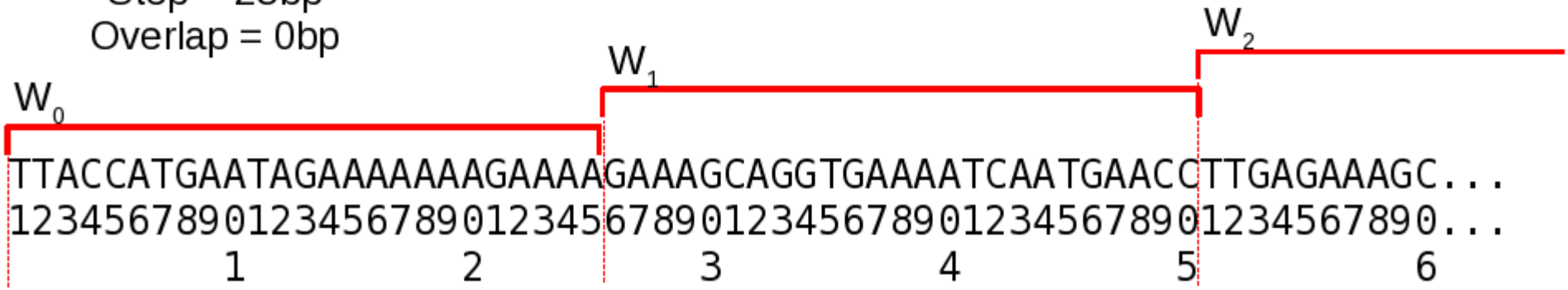
Zhang et al, *PNAS*, 2004

RUNNING WINDOWS

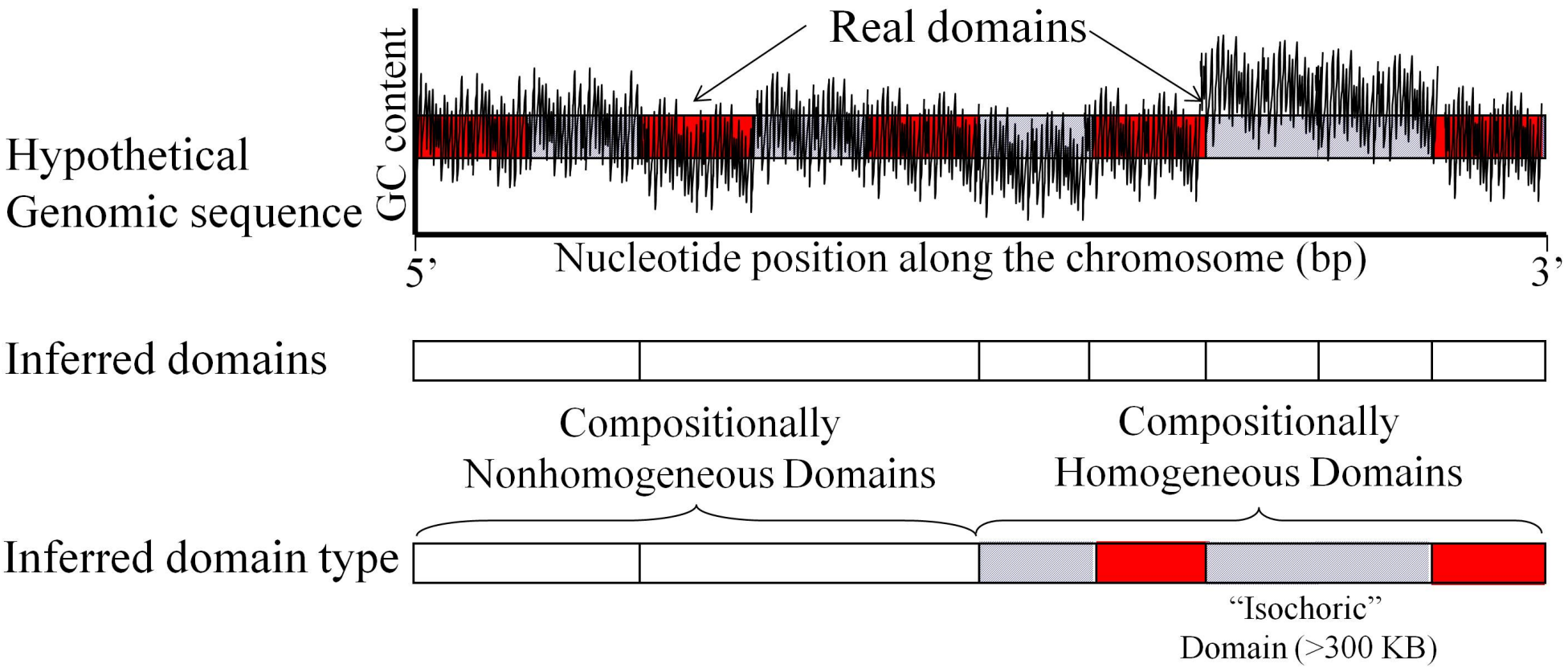
Window
Length = 25bp
Step = 20bp
Overlap = 5bp



Window
Length = 25bp
Step = 25bp
Overlap = 0bp

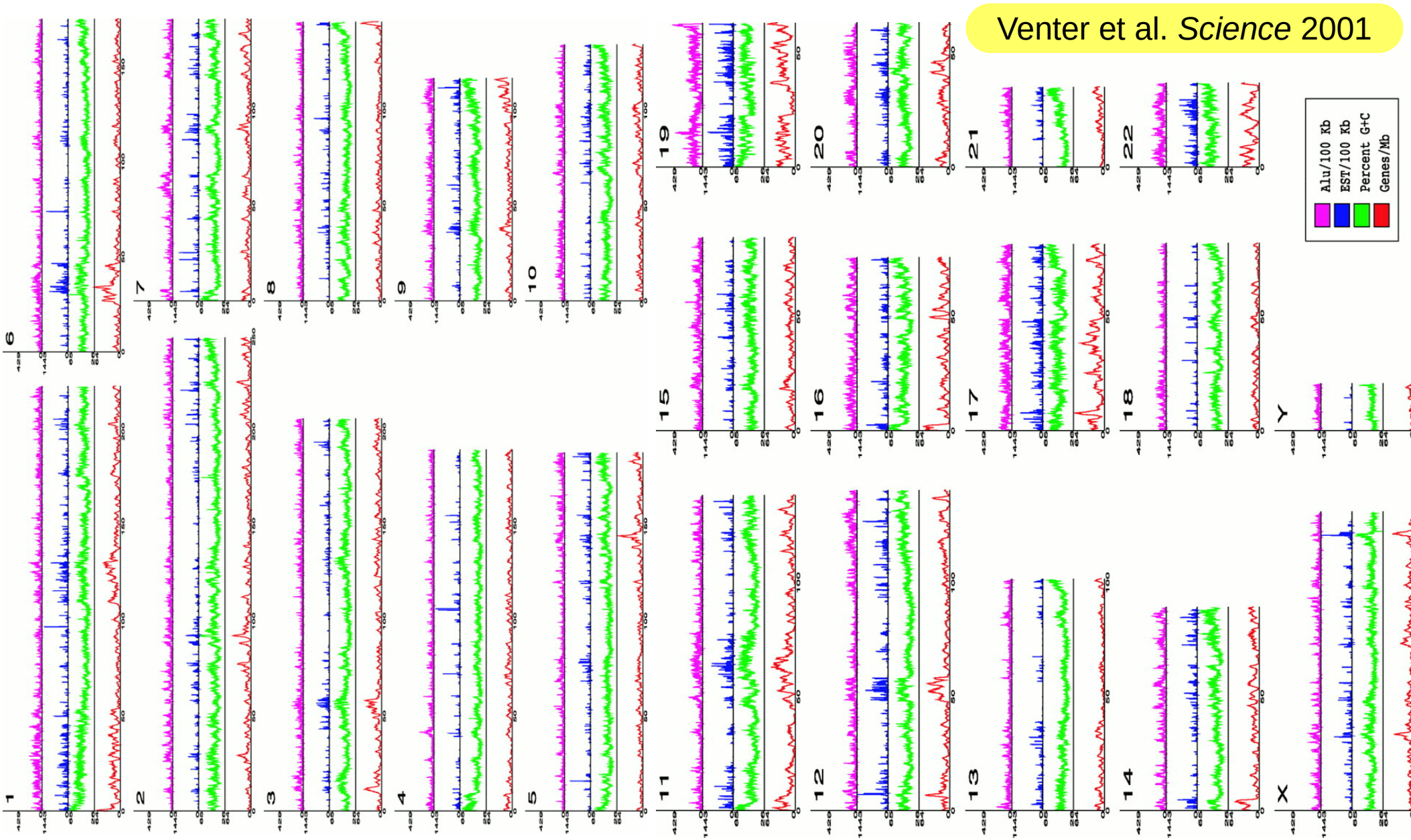


Genomic GC Content Variation

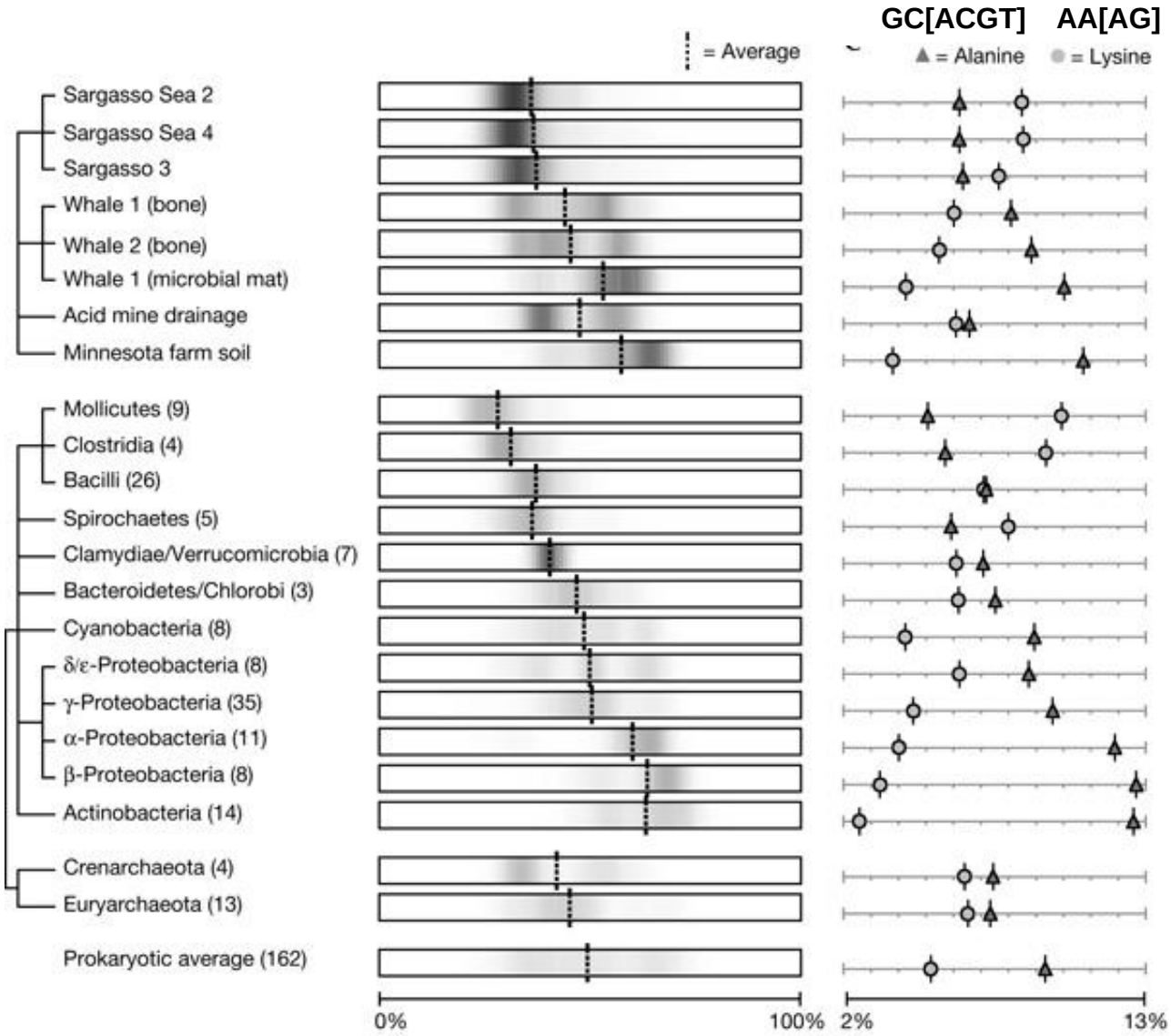


Genome-wide %GC Variation

Venter et al. *Science* 2001

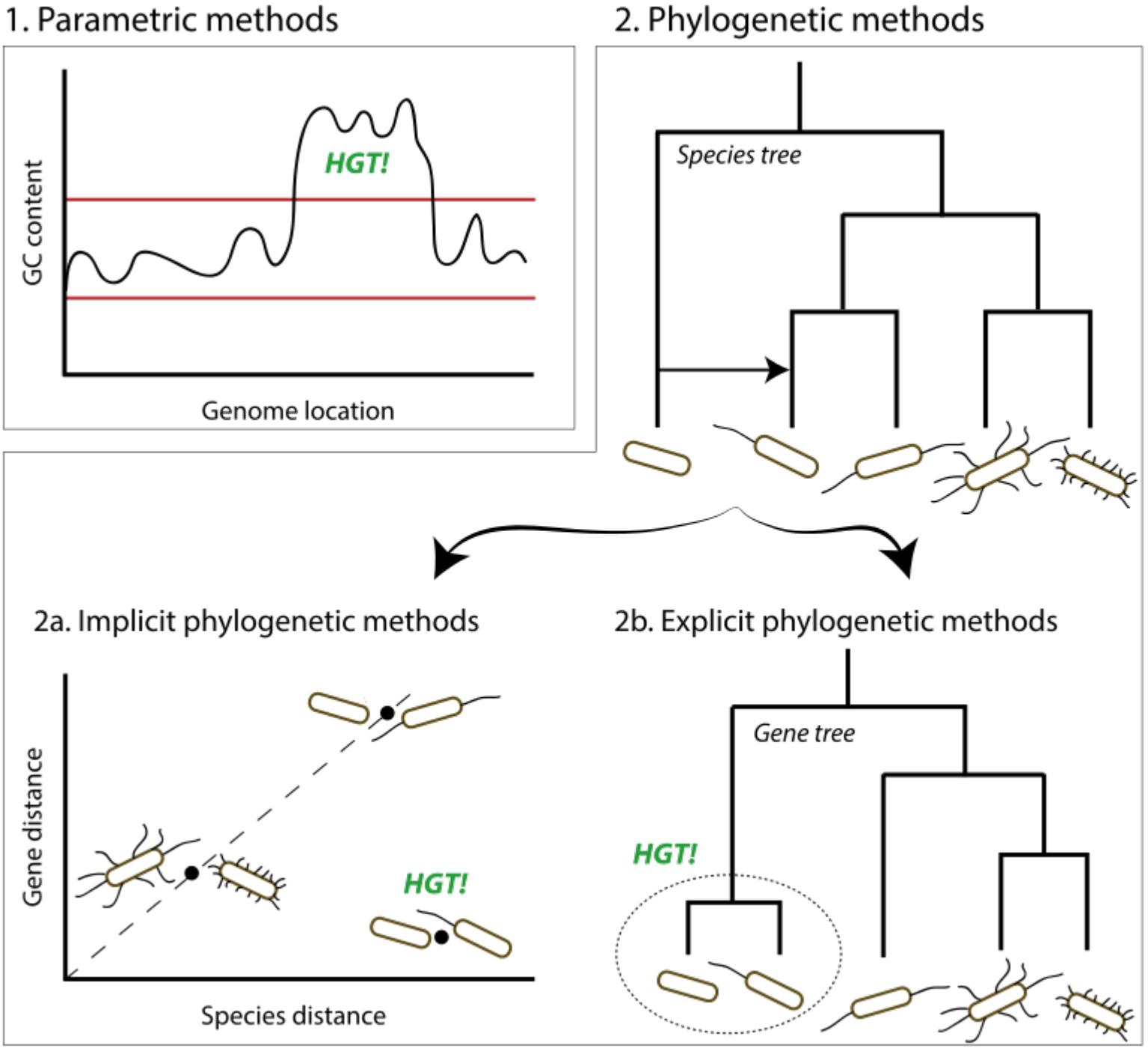


%GC and AA Composition



Foestner et al. *EMBO Rep*. 2005

%GC to Detect HGT Events



Ravenhall et a, PLoS Comput Bio, 2015

Strings of Symbols

We define an **alphabet** Σ, as a finite set of individual symbols:

$$\Sigma_{DNA.\,IUPAC} = \{ a, c, g, t, u, r, y, m, k, w, s, b, d, h, v, n \}$$

$$\Sigma_{AminoAcids} = \{ a, r, n, d, c, q, e, g, h, i, l, k, m, f, p, s, t, w, y, v, b, z, x \}$$

A **string** S is an ordered list of contiguous symbols taken from an alphabet; where $|S|$ denotes the string **length**, the number of characters in string S.

$$S = \left(\alpha_1, \ldots, \alpha_{|S|} \right); \quad \forall i \in [1, |S|]; \ \alpha_i \in \Sigma$$

A **substring** $S[i..j]$ is a contiguous set of symbols from S, starting at position i and ending at position j of S. In particular:

$$S[1..i], \text{ where } i \leq |S|, \text{ is the } \textbf{prefix} \text{ of string } S; \text{ and}$$

$$S[i..|S|], \text{ where } 1 \leq i, \text{ is the } \textbf{suffix} \text{ of string } S$$

Sequences as Strings

Given an **alphabet** for nucleotide sequences:

$$\Sigma_{DNA} = \{ a, c, g, t \}$$

A nucleotide **sequence** can be then modeled as a string of symbols taken from the DNA alphabet with a given length *n*.

$$seq_{DNA} = \left(\alpha_1, \ldots, \alpha_n \right); \ \forall i \in [1, n]; \ \alpha_i \in \Sigma_{DNA}$$

A ***k*-mer** can be understood as a word or a substring within such sequence string, having a fixed length of *k* symbols (say here nucleotides).

$$kmer_{DNA} = \left(\beta_1, \ldots, \beta_k \right);$$

$$\forall j \in [1, k]; k \leq n; \ \beta_j \in \Sigma_{DNA}; \ kmer_{DNA} \subset seq_{DNA}$$