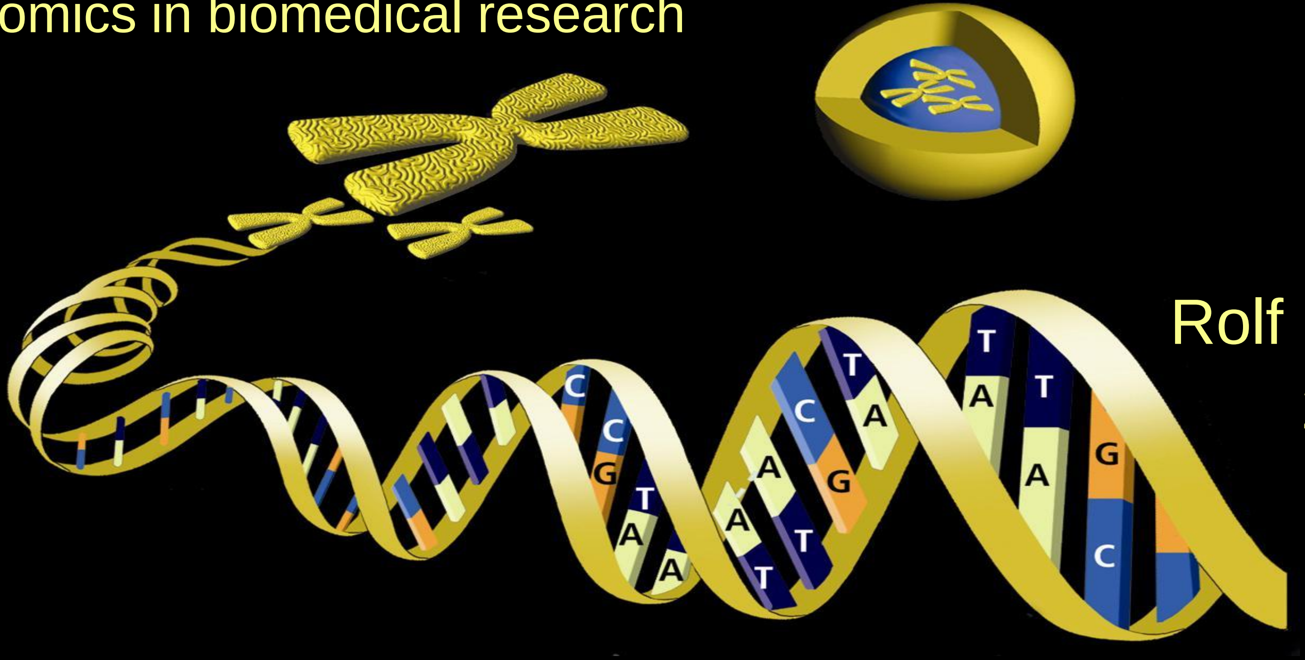
Fundamentals of molecular biology

IN-BIOS 5000/9000

1. A guided tour of the (human) genome
2. From DNA to biological function
3. Genomics in biomedical research



Rolf I. Skotheim

28.10.2024

A guided tour of

the (human) genome

Basic biology incl brief history of genetics

and genome sequencing

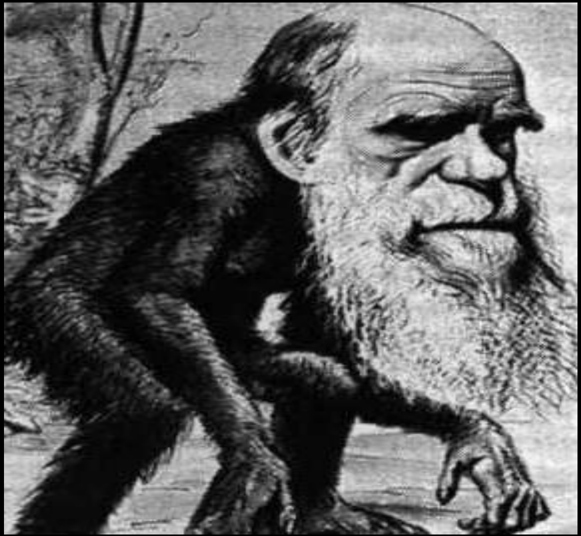
Early days of genetics (unaware of DNA)

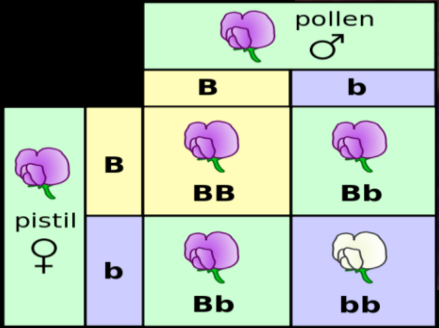
Breeding and selection

Early days of genetics (unaware of DNA)

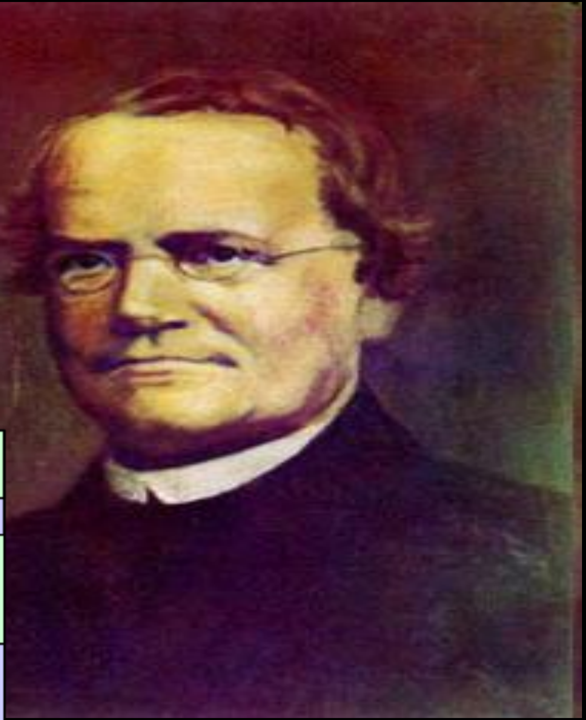
Gregor Mendel

Charles Darwin



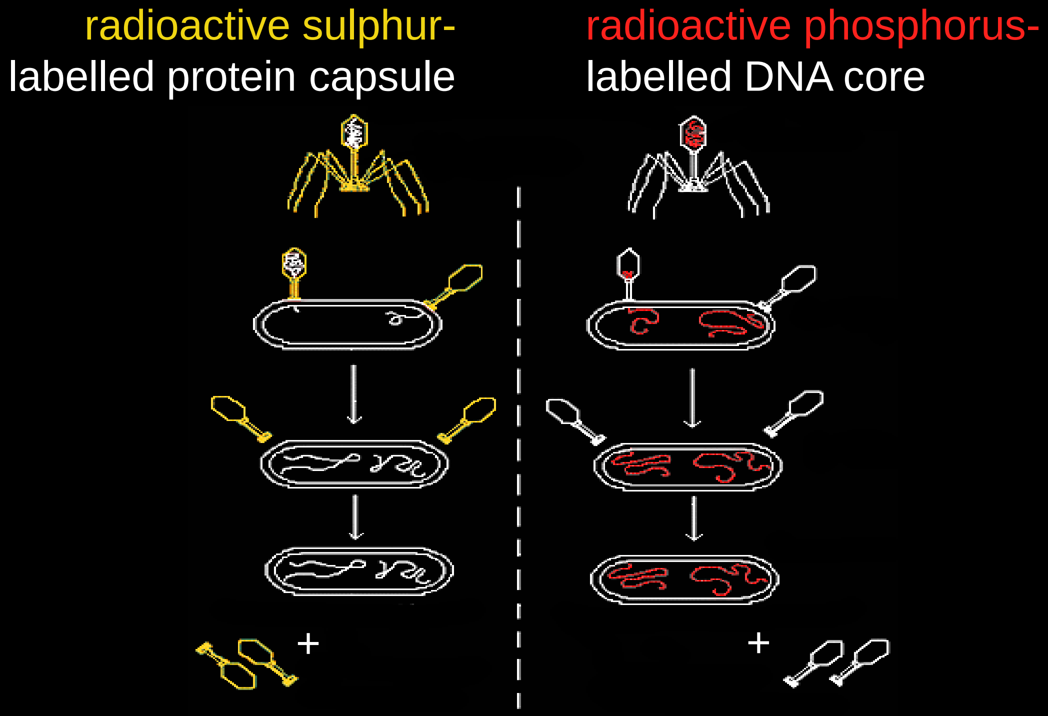


		pollen ♂	
		B	b
pistil ♀	B	BB	Bb
	b	Bb	bb



Early days of genetics (unaware of DNA)

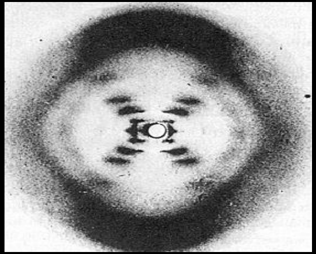
Proof that genes are made of DNA

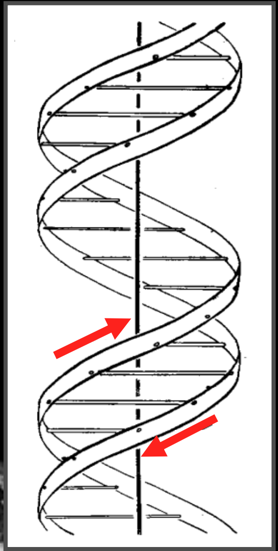


Hershey & Chase, 1952

1953: The DNA double helix

- Double helix
- Bidirectional





No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

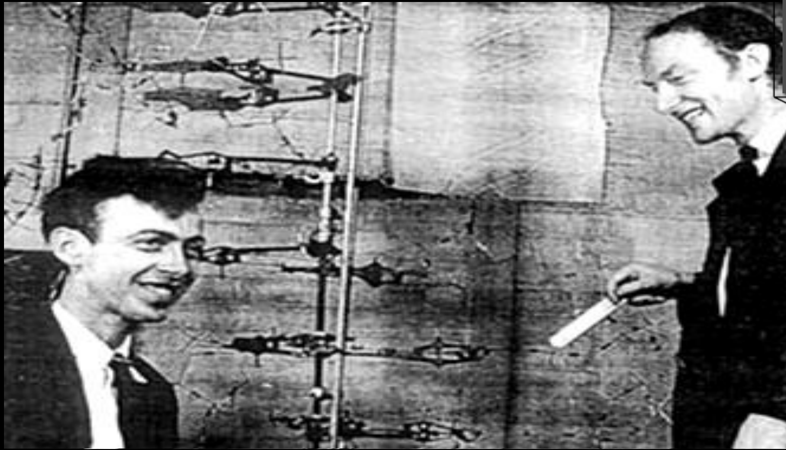
It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

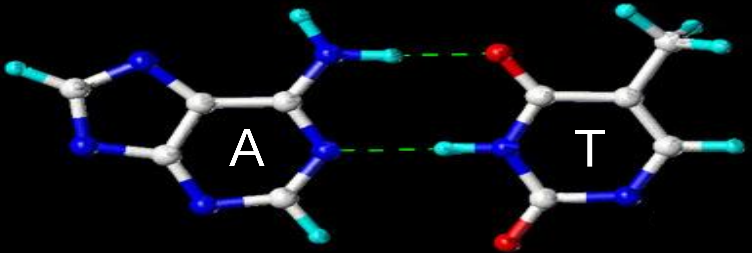
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

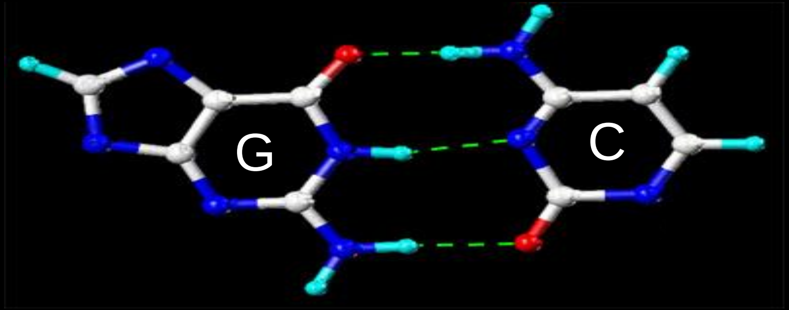


Watson & Crick
Wilkins, Stokes, & Wilson
Franklin & Gosling

1953: The DNA double helix

- Double helix
- Bidirectional
- Base-specific pairing





Watson & Crick
Wilkins, Stokes, & Wilson
Franklin & Gosling

The central dogma

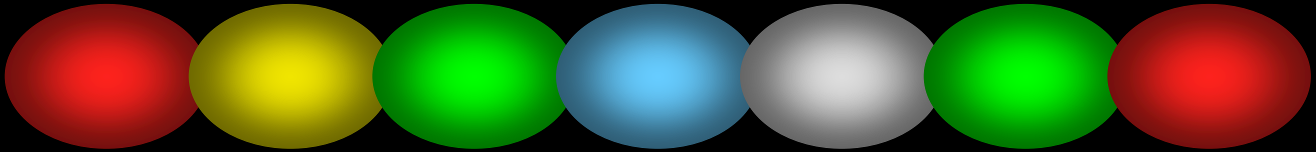
DNA ACGTCCATGCAGGATATGACG

↓

RNA ACGUCCAUGCAGGAUAUGACG

↓

Protein



The genetic code

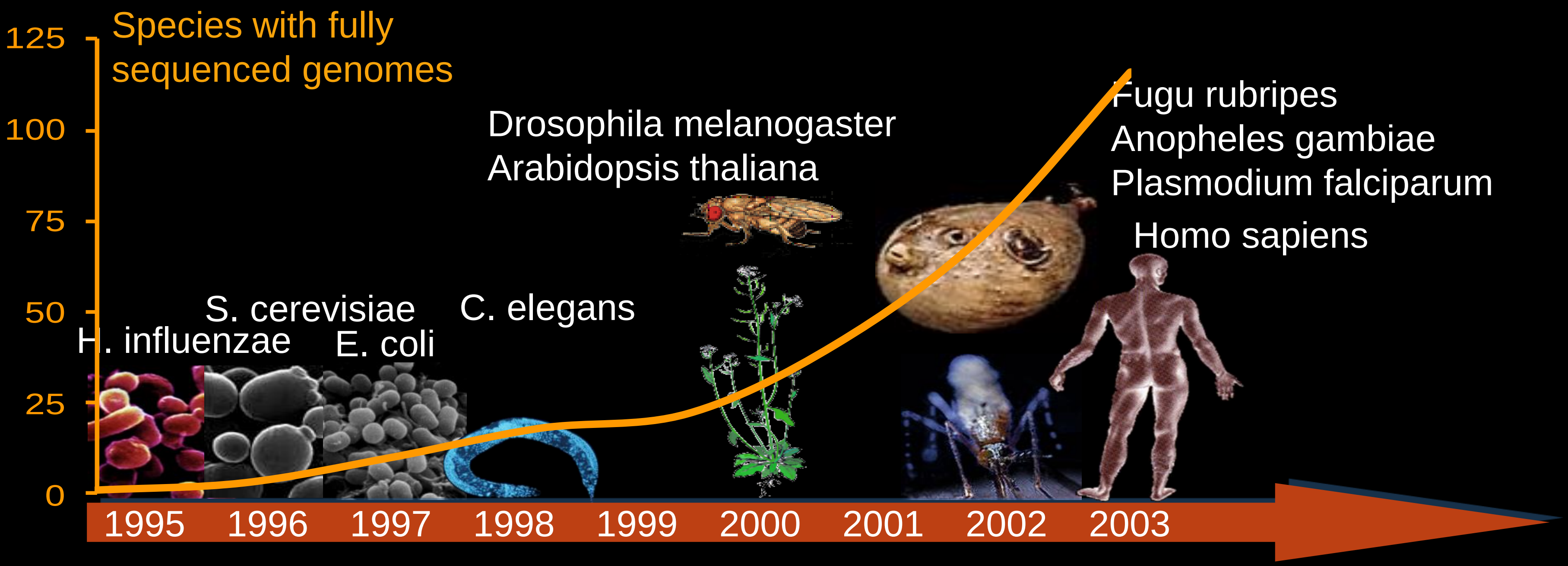
		2									
		U		C		A		G			
1	U	UUU UUC	Phe	UCU UCC	Ser	UAU UAC	Tyr	UGU UGC	Cys	U C	3
		UUA UUG	Leu	UCA UCG	Ser	UAA UAG	Stop	UGA UGG	Stop Trp	A G	
	C	CUU CUC	Leu	CCU CCC	Pro	CAU CAC	His	CGU CGC	Arg	U C	
		CUA CUG	Leu	CCA CCG	Pro	CAA CAG	Gln	CGA CGG	Arg	A G	
	A	AUU AUC	Ile	ACU ACC	Thr	AAU AAC	Asn	AGU AGC	Ser	U C	
		AUA AUG	Met	ACA ACG	Thr	AAA AAG	Lys	AGA AGG	Arg	A G	
	G	GUU GUC	Val	GCU GCC	Ala	GAU GAC	Asp	GGU GGC	Gly	U C	
		GUA GUG	Val	GCA GCG	Ala	GAA GAG	Glu	GGA GGG	Gly	A G	

A guided tour of

the human genome

Basic biology incl brief history of genetics and genome sequencing

Some early sequenced genomes



140 400

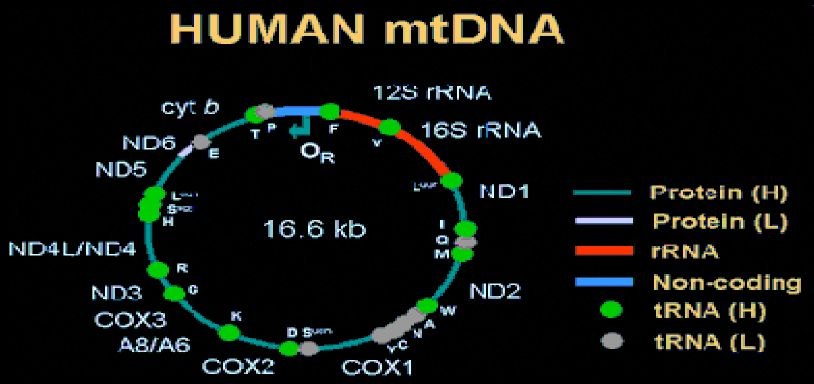
Genome sizes
(Mbp): 2 13 5 100 100 275/25 3200

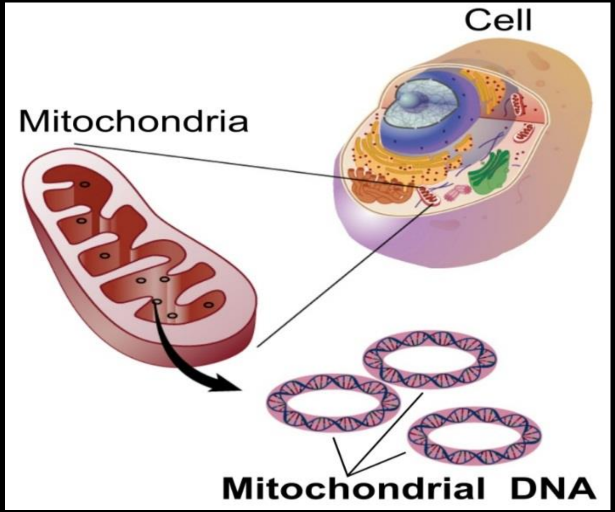
The human genome in numbers

- ~6.4 billion basepairs on 46 linear DNA molecules
  - (2 x 23 chromosomes [22 auto-chromosomes and X, Y sex chromosomes])

The human genome in numbers

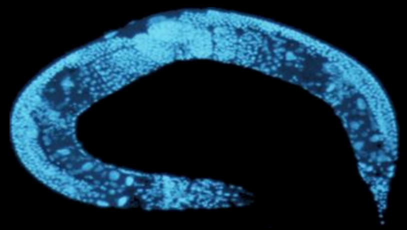
- ~6.4 billion basepairs on 46 linear DNA molecules
- Mitochondrion: circular DNA molecule of 16 569 bp





The human genome in numbers

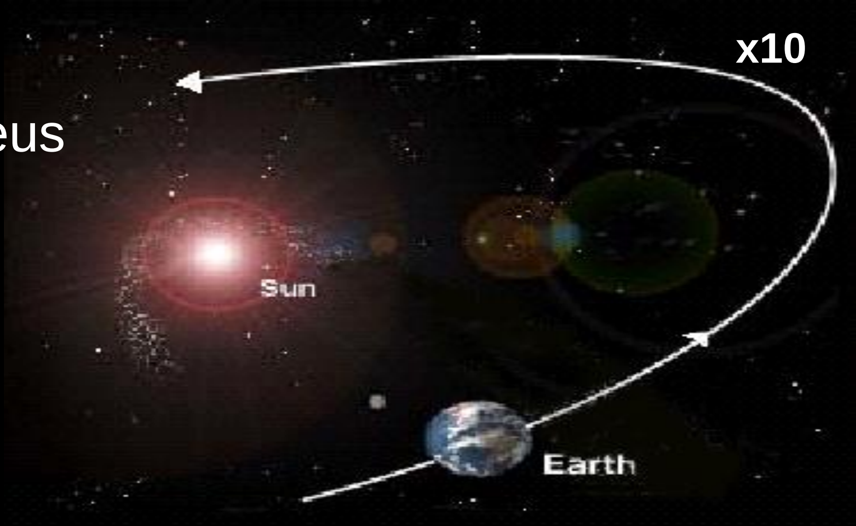
- ~6.4 billion basepairs on 46 linear DNA molecules
- Mitochondrion: circular DNA molecule of 16 569 bp
- 20 000 protein coding genes



The human genome in numbers

- ~6.4 billion basepairs on 46 linear DNA molecules
- Mitochondrion: circular DNA molecule of 16 569 bp
- 20 000 protein coding genes
- Two meters DNA in each cell nucleus

  (10 billion km in an adult human)

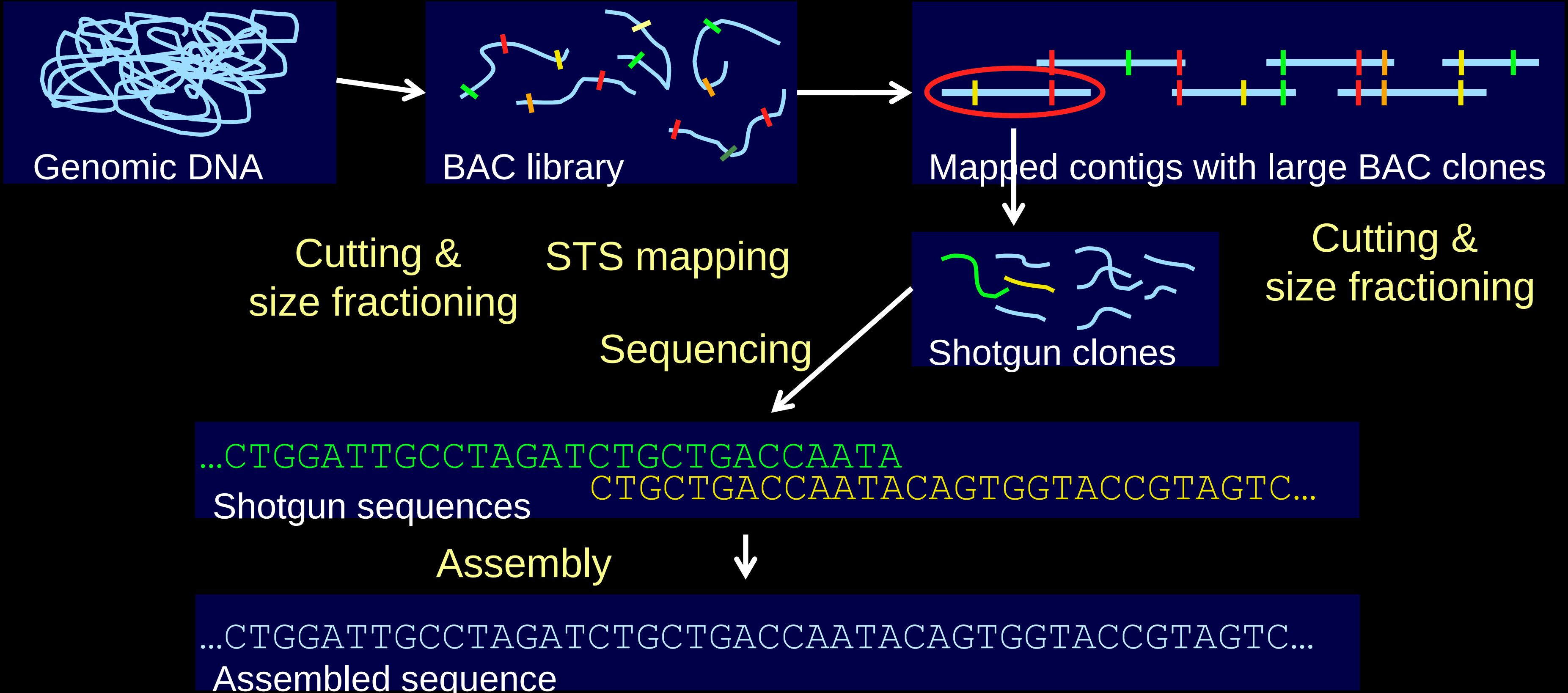


The human genome in numbers

- ~6.4 billion basepairs on 46 linear DNA molecules
- Mitochondrion: circular DNA molecule of 16 569 bp
- 20 000 protein coding genes
- Two meters DNA in each cell nucleus
- 3 billion US $

Human
Genome
Project

How to sequence a genome (historic)



Genomic DNA

Cutting & size fractioning

BAC library

STS mapping

Mapped contigs with large BAC clones

Cutting & size fractioning

Shotgun clones

Sequencing

...CTGGATTGCCTAGATCTGCTGACCAATA

CTGCTGACCAATACAGTGGTACCGTAGTC...

Shotgun sequences

Assembly

...CTGGATTGCCTAGATCTGCTGACCAATACAGTGGTACCGTAGTC...

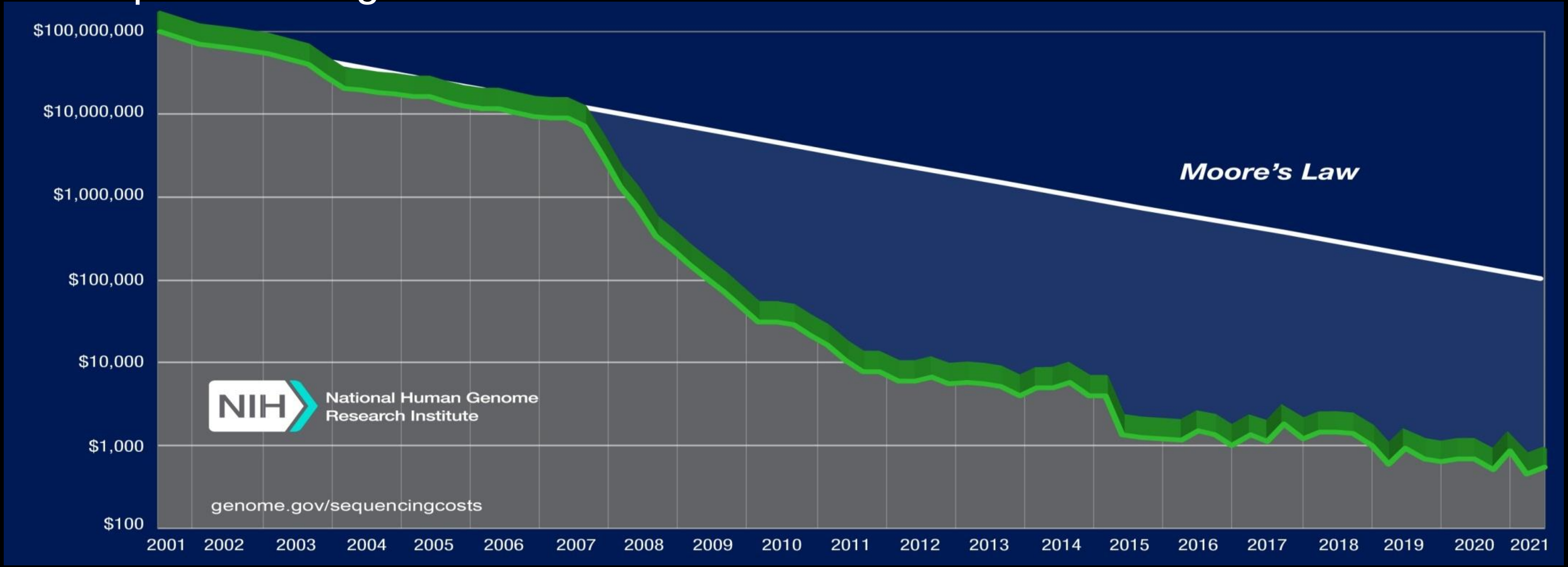
Assembled sequence

Genome sequencing, then and now

Year 2000, announcement of the first human genome sequence

Genome sequencing, then and now

Cost per human genome



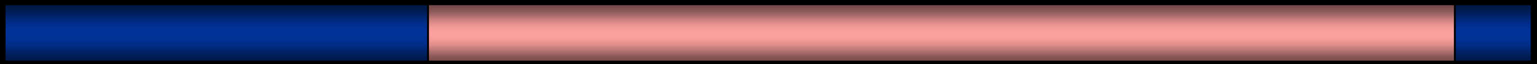
Main current («Next-generation») genome sequencing (NGS) technologies

- Short-read sequencing (Illumina)
- Long-read sequencing (Pacific Biosciences, Oxford Nanopore Technologies)

Sequence annotation

AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACCACGCGTTCACGAATACGC
GCTACGTCAACGACGACGATACGCGCGCGTCGCGACGACGTCGTGCGACGACGCTACGTCGAAATACGC
GCGCGTCGCGAACGTACGTCGCGACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTC
AAATATATAAGGCGTCGACGTTGCACCACGCTTCTCAAGCGCTACCAATAGGCGTCGAACGTTGCACCACG
CTTCAAATATGCGTCGAACGTTGCACCACGCTGAGGTAAGTCGAATAGGCGTCGAACGTTGCACCACGCT
ACGTCAATAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTTCAAA
TAGGCGTCGAACGTTGCACCATCCTTCACAGCGCTTCAAATAGGCGTCGAACGTTGCACCACGCTTCAAAT
AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACACGCTTCAAATAGGCGTCG
AACGTACGTGCACCACGCTTCAAGGTAAGTAATAGGCGTCGAACGTTGCACCACGCTACGTCAAATAGGC
GTCGAACGTACGTGCACCACGCTACGTCAACGAATAGGCGTCGAACGTTGCACCACGCTACGTCAATAGG
CGTCGAACGTTGCACCACGCTTCACGAACGAATAGGCACGGTCGAACGTACGTGCACCACGCTTCAAATA
GGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTCAAATCCTTC
ACAGTAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTTGCACACGCTTCAAATAAGGCG
TCGAACGTTGCACCACGAGGTAAGTCTACGTCAAACGATAGGCGTCGAACGTACGTGCACCACG

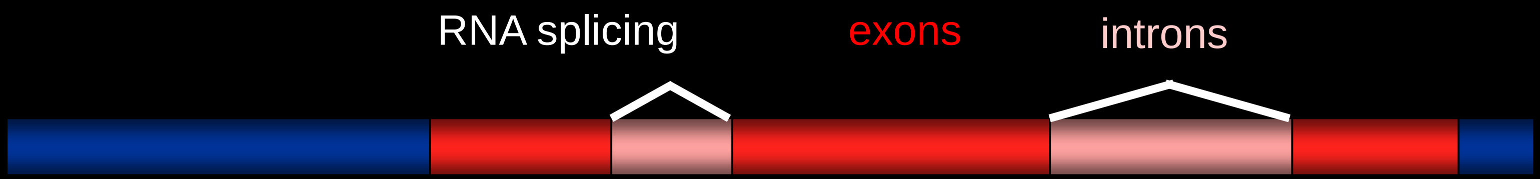
Sequence annotation



The gene (pre-mRNA)

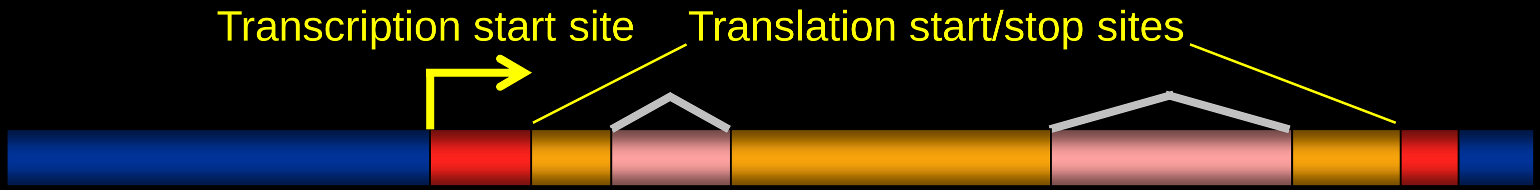
AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACCACGCGTTCACGAATACGC
GCTACGTCAACGACGACGATACGCGCGCGTCGCGACGACGTCGTGCGACGACGCTACGTCGAAATACGC
GCGCGTCGCGAACGTACGTCGCGACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTC
AAATATATAAGGCGTCGACGTTGCACCACGCTTCTCAAGCGCTACCAATAGGCGTCGAACGTTGCACCACG
CTTCAAATATGCGTCGAACGTTGCACCACGCTGAGGTAAGTCGAATAGGCGTCGAACGTTGCACCACGCT
ACGTCAATAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTTCAAA
TAGGCGTCGAACGTTGCACCATCCTTCACAGCGCTTCAAATAGGCGTCGAACGTTGCACCACGCTTCAAAT
AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACACGCTTCAAATAGGCGTCG
AACGTACGTGCACCACGCTTCAAGGTAAGTAATAGGCGTCGAACGTTGCACCACGCTACGTCAAATAGGC
GTCGAACGTACGTGCACCACGCTACGTCAACGAATAGGCGTCGAACGTTGCACCACGCTACGTCAATAGG
CGTCGAACGTTGCACCACGCTTCACGAACGAATAGGCACGGTCGAACGTACGTGCACCACGCTTCAAATA
GGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTCAAATCCTTC
ACAGTAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTTGCACACGCTTCAAATAAGGCG
TCGAACGTTGCACCACGAGGTAAGTCTACGTCAAACGATAGGCGTCGAACGTACGTGCACCACG

Sequence annotation



AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACCACGCGTTCACGAATACGC
GCTACGTCAACGACGACGATACGCGCGCGTCGCGACGACGTCGTGCGACGACGCTACGTCGAAATACGC
GCGCGTCGCGAACGTACGTCGCGACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTC
AAATATATAAGGCGTCGACGTTGCACCACGCTTCTCAAGCGCTACCAATAGGCGTCGAACGTTGCACCACG
CTTCAAATATGCGTCGAACGTTGCACCACGCTGAGGTAAGTCGAATAGGCGTCGAACGTTGCACCACGCT
ACGTCAATAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTTCAAA
TAGGCGTCGAACGTTGCACCATCCTTCACAGCGCTTCAAATAGGCGTCGAACGTTGCACCACGCTTCAAAT
AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACACGCTTCAAATAGGCGTCG
AACGTACGTGCACCACGCTTCAAGGTAAGTAATAGGCGTCGAACGTTGCACCACGCTACGTCAAATAGGC
GTCGAACGTACGTGCACCACGCTACGTCAACGAATAGGCGTCGAACGTTGCACCACGCTACGTCAATAGG
CGTCGAACGTTGCACCACGCTTCACGAACGAATAGGCACGGTCGAACGTACGTGCACCACGCTTCAAATA
GGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTCAAATCCTTC
ACAGTAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTTGCACACGCTTCAAATAAGGCG
TCGAACGTTGCACCACGAGGTAAGTCTACGTCAAACGATAGGCGTCGAACGTACGTGCACCACG

Sequence annotation

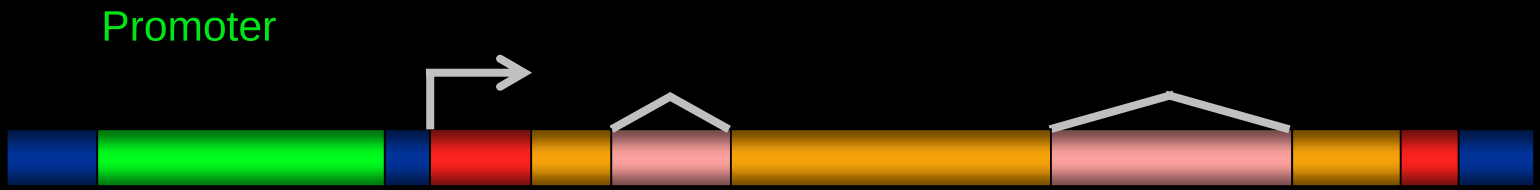


Transcription start site

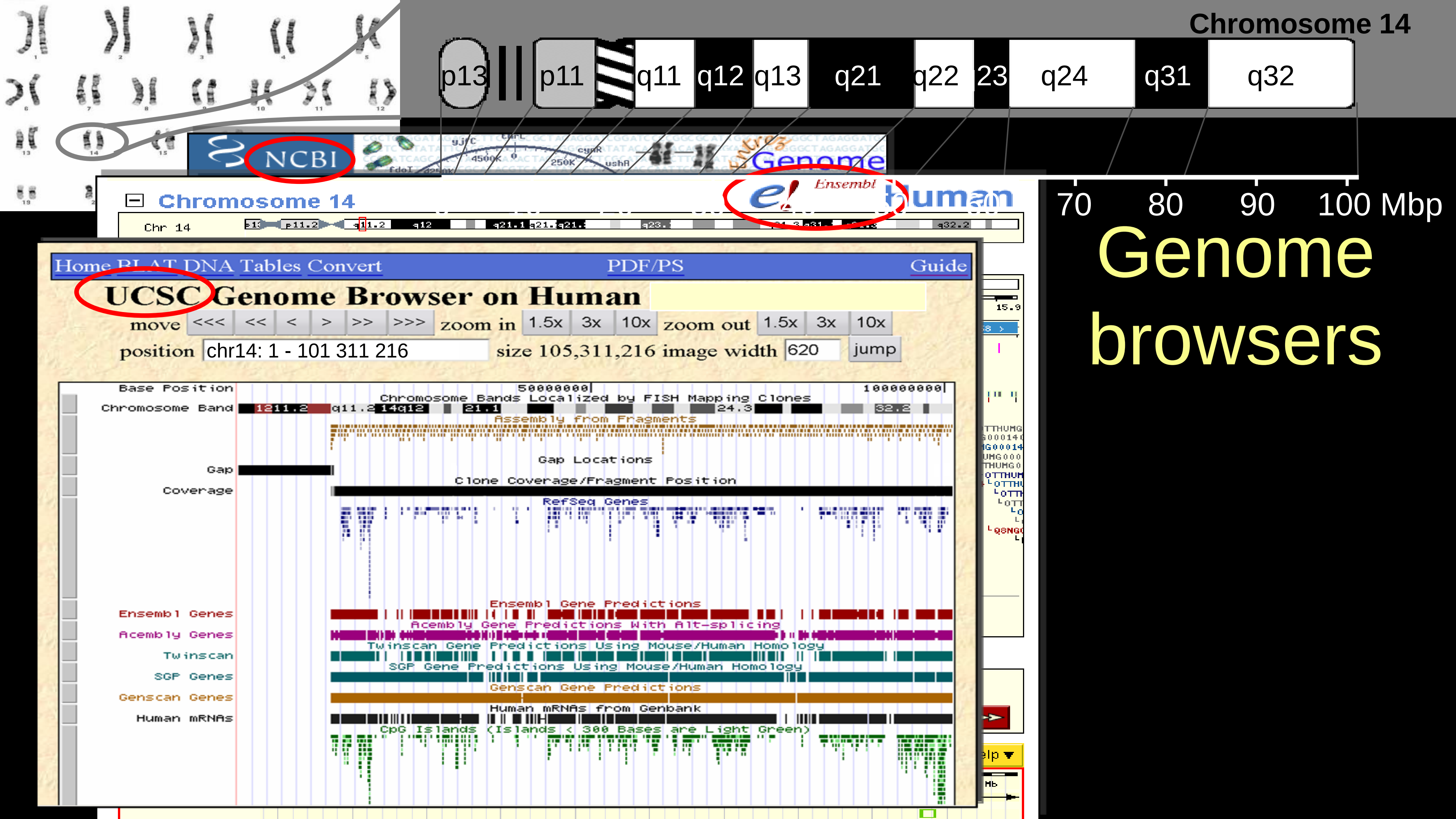
Translation start/stop sites

AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACCACGCGTTCACGAATACGC
GCTACGTCAACGACGACGATACGCGCGCGTCGCGACGACGTCGTGCGACGACGCTACGTCGAAATACGC
GCGCGTCGCGAACGTACGTCGCGACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTC
AAATATATAAGGCGTCGACGTTGCACCACGCTTCTCAAGCGCTACCAATAGGCGTCGAACGTTGCACCACG
CTTCAAAT**ATG**CGTCGAACGTTGCACCACGCTGAGGTAAGTCGAATAGGCGTCGAACGTTGCACCACGCT
ACGTCAATAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTTCAAA
TAGGCGTCGAACGTTGCACCATCCTTCACAGCGCTTCAAATAGGCGTCGAACGTTGCACCACGCTTCAAAT
AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACACGCTTCAAATAGGCGTCG
AACGTACGTGCACCACGCTTCAAGGTAAGTAATAGGCGTCGAACGTTGCACCACGCTACGTCAAATAGGC
GTCGAACGTACGTGCACCACGCTACGTCAACGAATAGGCGTCGAACGTTGCACCACGCTACGTCAATAGG
CGTCGAACGTTGCACCACGCTTCACGAACGAATAGGCACGGTCGAACGTACGTGCACCACGCTTCAAATA
GGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTCAAATCCTTC
ACAGTAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTTGCACACGCTTCAAA**TAA**GGCG
TCGAACGTTGCACCACGAGGTAAGTCTACGTCAAACGATAGGCGTCGAACGTACGTGCACCACG

Sequence annotation



AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACCACGCGTTCACGAATACGC
GCTACGTCAACGACGACGATACGCGCGCGTCGCGACGACGTCGTGCGACGACGCTACGTCGAAATACGC
GCGCGTCGCGAACGTACGTCGCGACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTC
AAATATATAAGGCGTCGACGTTGCACCACGCTTCTCAAGCGCTACCAATAGGCGTCGAACGTTGCACCACG
CTTCAAATATGCGTCGAACGTTGCACCACGCTGAGGTAAGTCGAATAGGCGTCGAACGTTGCACCACGCT
ACGTCAATAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTTCAAA
TAGGCGTCGAACGTTGCACCATCCTTCACAGCGCTTCAAATAGGCGTCGAACGTTGCACCACGCTTCAAAT
AGGCGTCGAACGTTGCACCACGCTTCAACGAATAGGCGTCGAACGTTGCACACGCTTCAAATAGGCGTCG
AACGTACGTGCACCACGCTTCAAGGTAAGTAATAGGCGTCGAACGTTGCACCACGCTACGTCAAATAGGC
GTCGAACGTACGTGCACCACGCTACGTCAACGAATAGGCGTCGAACGTTGCACCACGCTACGTCAATAGG
CGTCGAACGTTGCACCACGCTTCACGAACGAATAGGCACGGTCGAACGTACGTGCACCACGCTTCAAATA
GGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTACGTGCACCACGCTACGTCAAATCCTTC
ACAGTAGGCGTCGAACGTTGCACCACGCTTCAAATAGGCGTCGAACGTTGCACACGCTTCAAATAAGGCG
TCGAACGTTGCACCACGAGGTAAGTCTACGTCAAACGATAGGCGTCGAACGTACGTGCACCACG



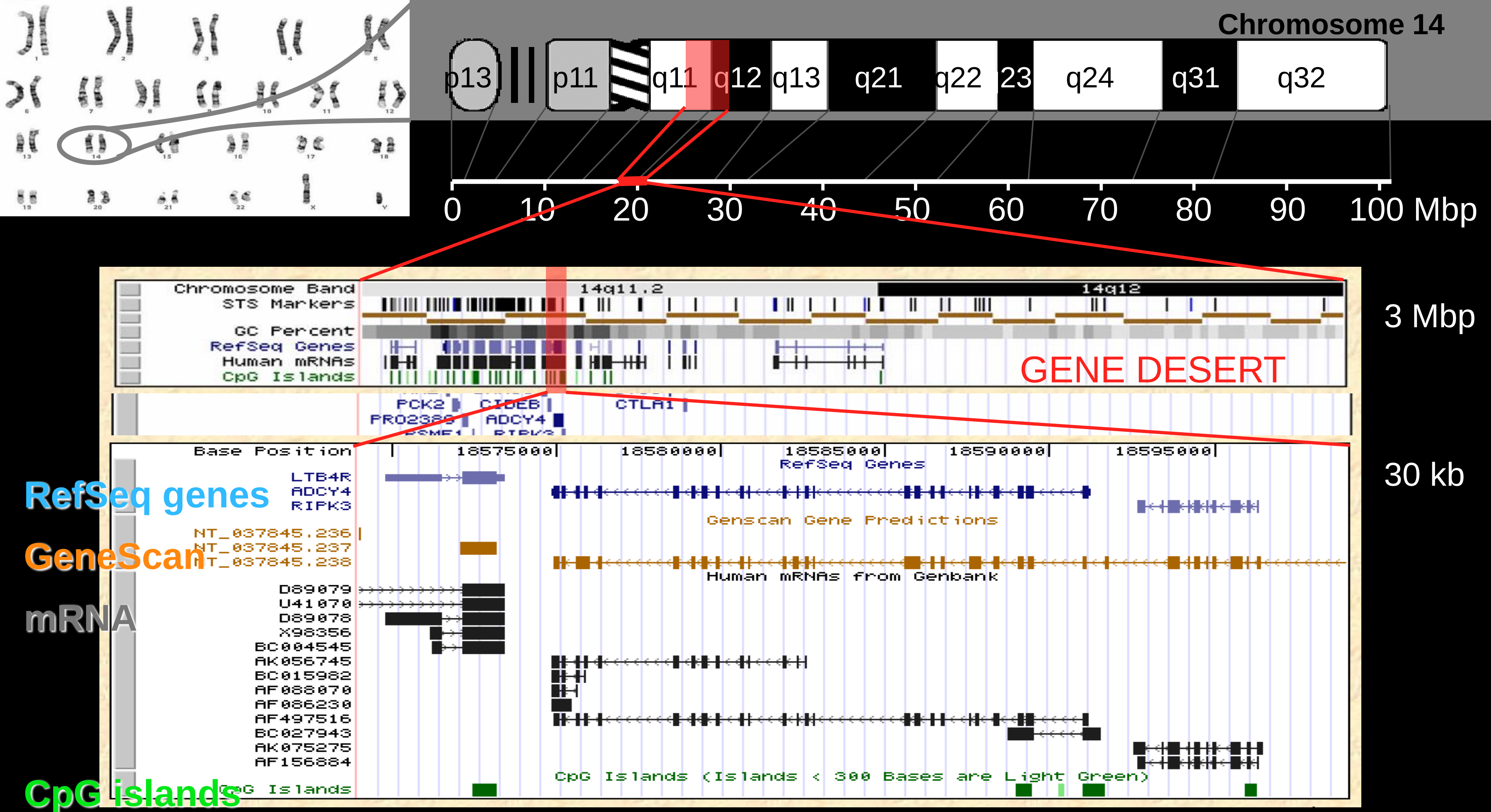
Home BLAT DNA Tables Convert PDF/PS Guide

UCSC Genome Browser on Human

move <<< << < > >> >>> zoom in 1.5x 3x 10x zoom out 1.5x 3x 10x

position chr14: 1 - 101 311 216 size 105,311,216 image width 620 jump

Genome browsers



Chromosome 14

3 Mbp

30 kb

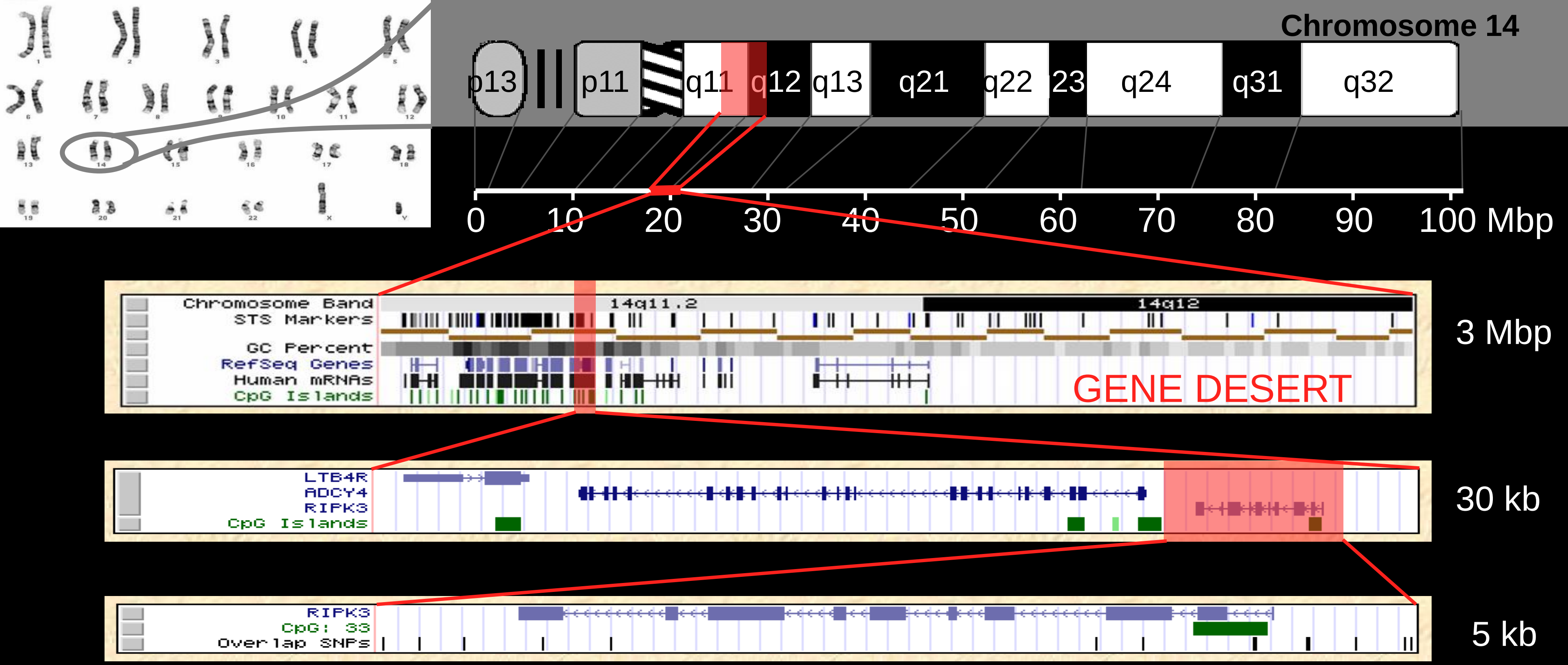
GENE DESERT

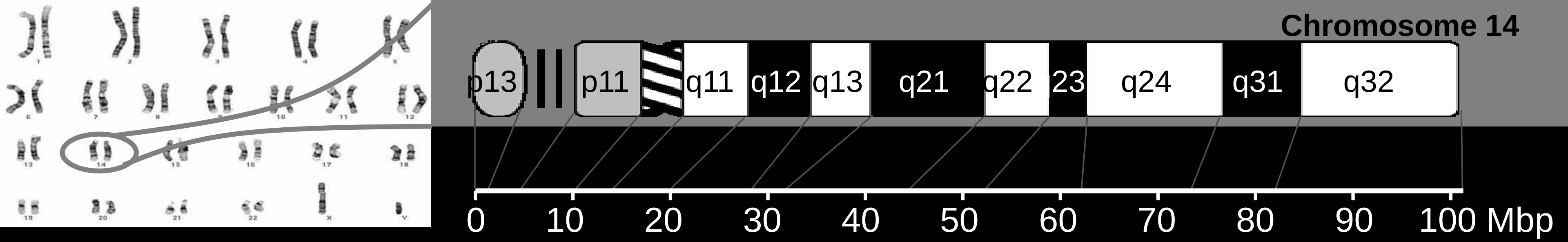
RefSeq genes

GeneScan

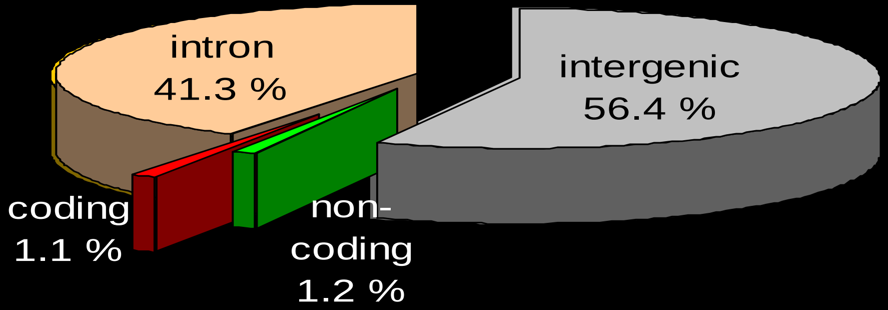
mRNA

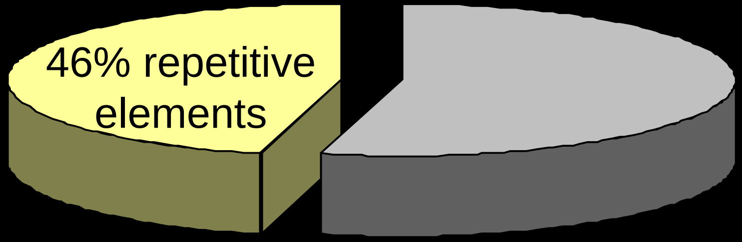
CpG islands


Chromosome 14
p13
p11
q11
q12
q13
q21
q22
23
q24
q31
q32
0
10
20
30
40
50
60
70
80
90
100 Mbp
Chromosome Band
14q11.2
14q12
STS Markers
GC Percent
RefSeq Genes
Human mRNAs
CpG Islands
GENE DESERT
3 Mbp
LTB4R
ADCY4
RIPK3
CpG Islands
30 kb
RIPK3
CpG: 33
Overlap SNPs
5 kb



- 1050 genes
- 1 gene / 100 kb





Gene ontology

Current release 2024-09-08:
43 939 GO terms | 7 894 411 annotations
1 573 444 gene products | 5 426 species
geneontology.org/

- Molecular function
- Biological process
- Cellular component

Gene ontology

Current release 2024-09-08:
43 939 GO terms | 7 894 411 annotations
1 573 444 gene products | 5 426 species
geneontology.org/

+ Molecular function
- Biological process
  + behaviour
  + cellular process
  + physiological process
  + viral life cycle
  + development
+ Cellular component

Gene ontology

Current release 2024-09-08:
43 939 GO terms | 7 894 411 annotations
1 573 444 gene products | 5 426 species
geneontology.org/

- \+ Molecular function
- \- Biological process
  - \+ behaviour
  - \- cellular process
    - \+ cell communication
    - \+ cell death
    - \+ cell differentiation
    - \+ cell motility
    - \+ membrane fusion
  - \+ physiological process
  - \+ viral life cycle
  - \+ development

Gene ontology

Current release 2024-09-08:
43 939 GO terms | 7 894 411 annotations
1 573 444 gene products | 5 426 species
geneontology.org/

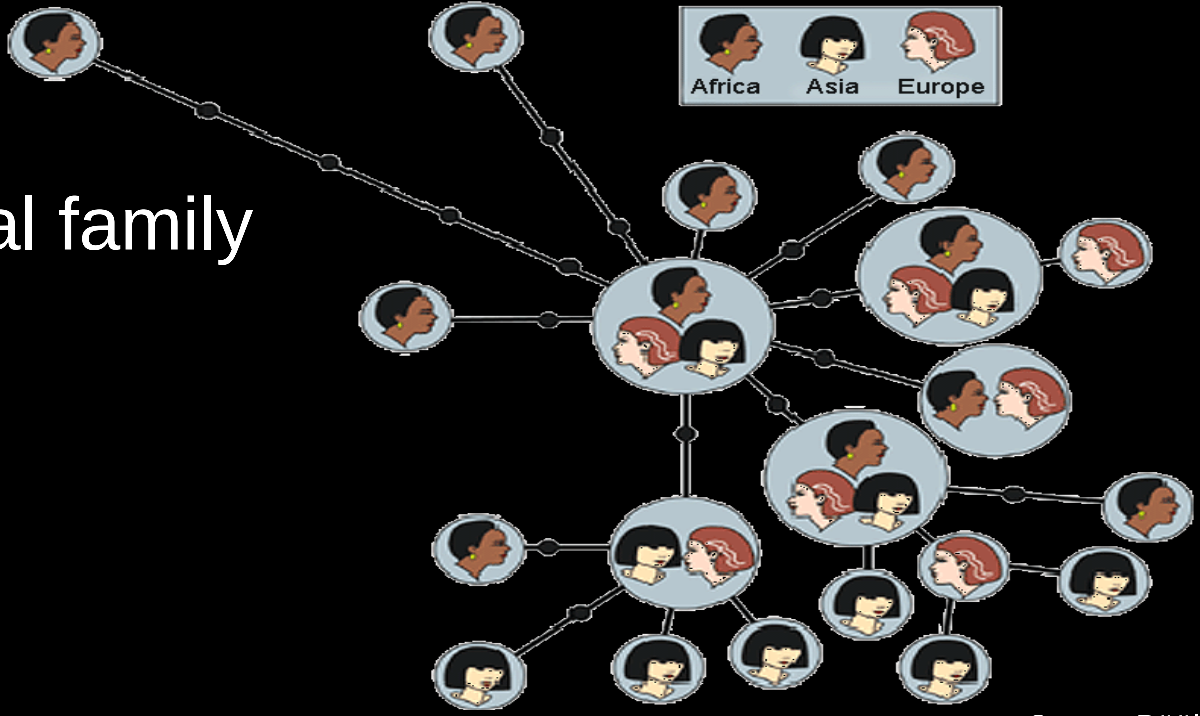
+ Molecular function
- Biological process
  + behaviour
  - cellular process
    - cell communication
      + cell adhesion
      + cell invasion
      + signal transduction
      + response to extra-cellular stimulus
      + cell-cell signalling
      + host-pathogen interaction
    + cell death
    + cell differentiation

DNA sequence variation

→ variant protein product?

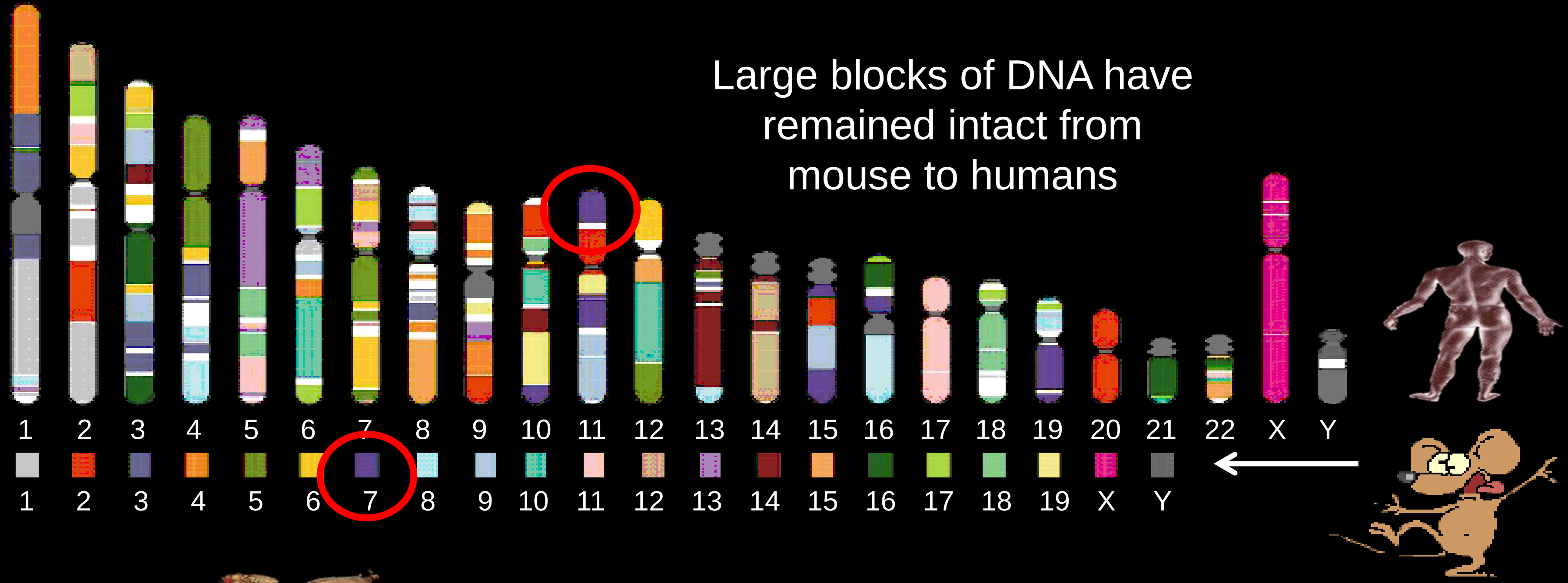
DNA sequence variation

The global family

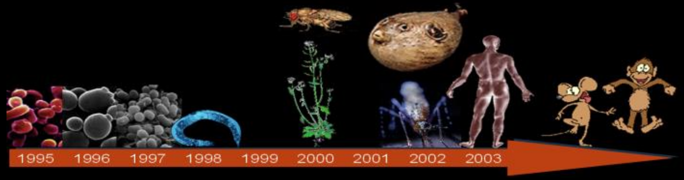


Svante Pääbo, Science 2001
Nobel Prize, 2022

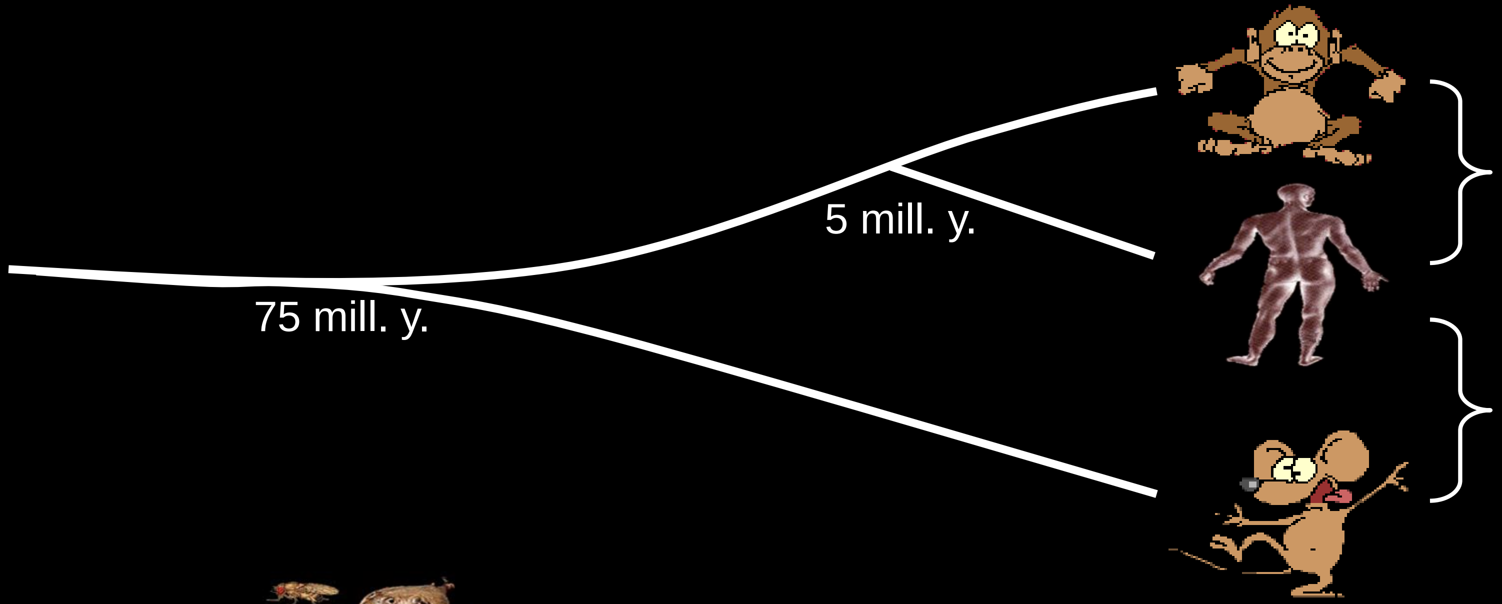
Comparative genomics



Large blocks of DNA have remained intact from mouse to humans



Comparative genomics



5 mill. y.

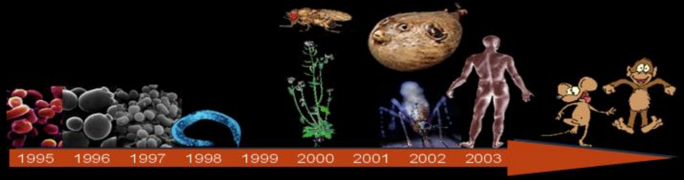
75 mill. y.

1 substitution

80 nucleotides

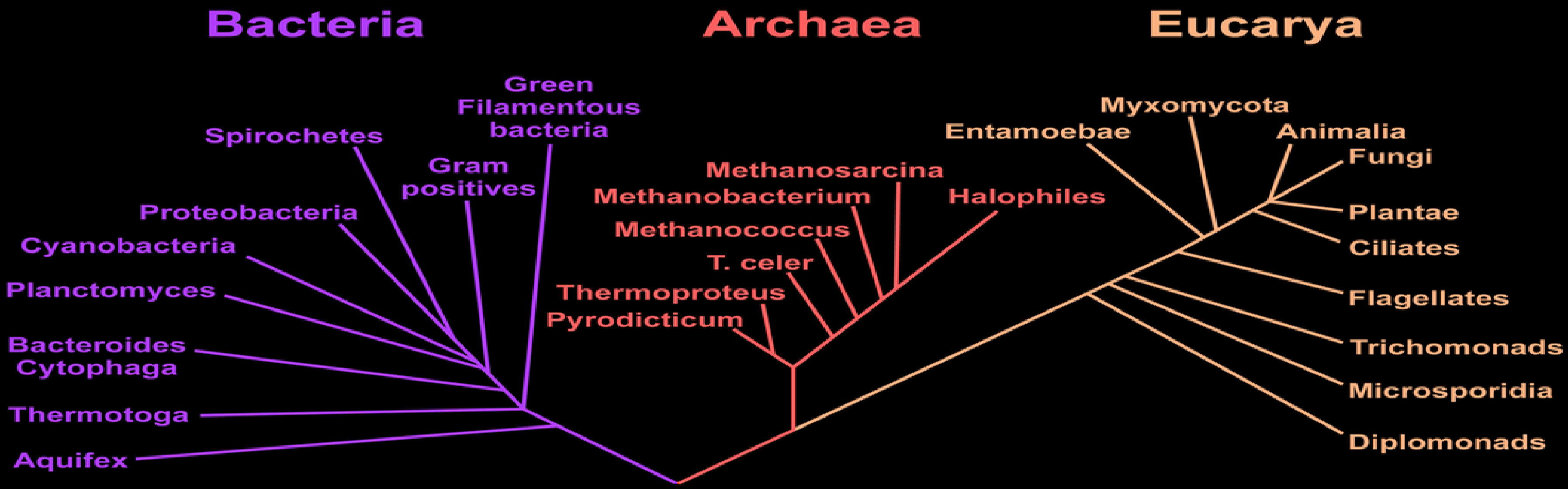
1 substitution

2 nucleotides



The phylogenetic tree of life

Taxonomy in biology. Asigning vectors (from aligned genomics data, commonly ribosomal-RNA) to species, calculating matrix of distances, group them with cluster analysis to obtain a tree or dendrogram



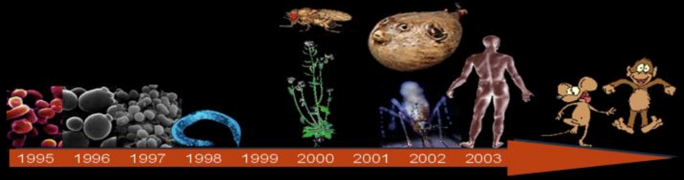
Eric Gaba

Comparative genomics

Distribution of gene-rich areas

human

non-human (*i.e.* most non-mammalian model organisms)



Comparative genomics

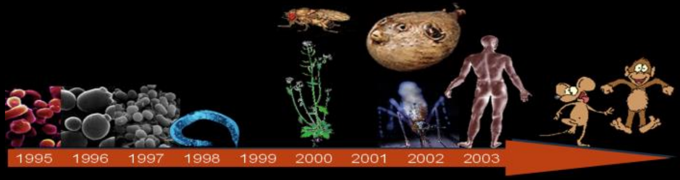
Repeat sequences

human

~ 50 %

non-human (*i.e.* most non-mammalian model organisms)

< 10 %

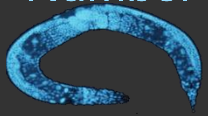


Comparative genomics

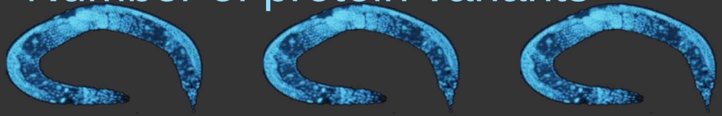
The human genome has many protein variants

human

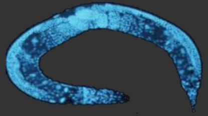
Number of genes

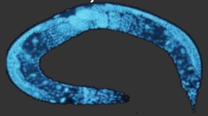


Number of protein variants



non-human (*i.e.* most non-mammalian model organisms; here: *c. elegans*)



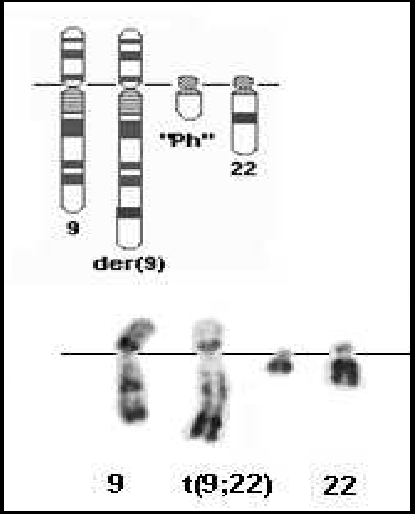


Gene regulation

- Time
- Space
- Level
- Alternative splicing
- Activity

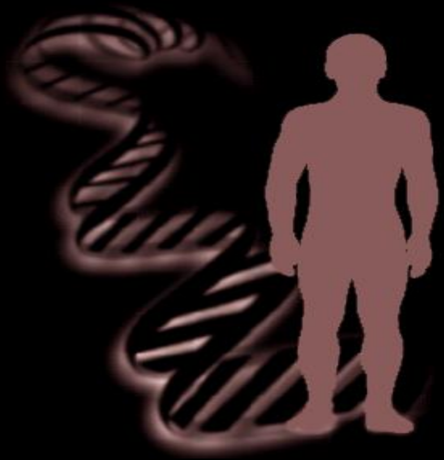
Genomics into clinics

- Individualise treatment
- Targeted and tailored “designer” medicine
  - Gleevec
  - t(9;22): Philadelphia chr.
  - Chronic myeloid leukaemia



Genomics into clinics

- Individualise treatment
- Targeted and tailored “designer” medicine
- High-throughput technologies
    - Primarily sequencing of DNA and RNA
        - DNA mutations/variation: base-level and larger
        - RNA expression: quantitative and qualitative
- Pre-symptomatic diagnosis (and genetic predisposition)
    - Huntington’s disease
    - Cystic fibrosis
    - Breast cancer
- Potential future health



Ethical, legal, and social implications