Computational gene finding in the human genome: how many genes do we have?

Steven Salzberg Director, Center for Bioinformatics and Computational Biology Horvitz Professor, Dept. of Computer Science University of Maryland http://cbcb.umd.edu

We've been trying for a long time to determine how many genes we have

letters to nature

Nature 201, 847 (22 February 1964); doi:10.1038/201847a0

A Preliminary Estimate of the Number of Human Genes

F. VOGEL

Institut für Anthropologie und Humangenetik, University of Heidelberg, Germany.

RECENT results of molecular genetics enable us to estimate the number of human genes, if certain assumptions are made. The following data are available: (1) The α -chain of human hæmoglobin contains 141, the _B-chain contains 146 amino-acids, corresponding to a molecular weight of about 17,000 each¹. Assuming a triplet code^{2,3} this means that the α - and _B-chains are determined by 423 and 438 nucleotide pairs, respectively. According to 'Svedberg's law'4, many proteins consist of sub-units of the same order of magnitude (molecular weight of about 17,500). Hence, the assumption seems to be warranted that one average structural geno might have a length of about 450 nucleotide pairs. (2) The weight of one haploid human chromosome set in human spermatozoa is about 2.72 × 10⁻¹² g. Granulocytes contain about 6.23 × 10⁻¹² g; lymphocytes contain about 5.84 × ⁻¹² g (ref. 5). Extensive examinations have shown that the DKA. content is constant in all resting cells of one species, which have the same number of chromosome sets, and depends on the degree of polyploidy^{5,6}. The assumption seems to be justified that most of the DNA works as genetic material, even if in some cells minor fractions with other functions might possibly be present⁷. In the following calculations the total amount of DNA in a haploid human chromosome set is estimated to be about 3 × 10⁻¹² g. (3) Usually the genetic variants of human haemoglobins differ in one amino-acid substitution only^{1,8}. One structural gene can only produce one single type of genetically determined polypeptide chain. As much as we know, this applies for other genetically determined proteins as well. This means that the genetic information for these structural genes can only be present or diploid cells is about twice the content of (haploid) spermatozoa. We assume that the total genetic information is only present once.

1,000,000 genes? 100,000 genes?

Science 25 October 1996: Vol. 274. no. 5287, pp. 540 - 546 DOI: 10.1126/science.274.5287.540 < Prev | Table of Contents | Next >

ARTICLES

A Gene Map of the Human Genome

G. D. Schuler, M. S. Boguski, E. A. Stewart, L. D. Stein, G. Gyapay, K. Rice, R. E. White, P. Rodriguez-Tomé, A. Aggarwal, E. Bajorek, S. Bentolila, B. B. Birren, A. Butler, A. B. Castle, N. Chiannilkulchai, A. Chu, C. Clee, S. Cowles, P. J. R. Day, T. Dibling, N. Drouot, I. Dunham, S. Duprat, C. East, C. Edwards, J.-B. Fan, N. Fang, C. Fizames, C. Garrett, L. Green, D. Hadley, M. Harris, P. Harrison, S. Brady, A. Hicks, E. Holloway, L. Hui, S. Hussain, C. Louis-Dit-Sully, J. Ma, A. MacGilvery, C. Mader, A. Maratukulam, T. C. Matise, K. B. McKusick, I. Morissette, A. Mungall, D. Muselet, H. C. Nusbaum, D. C. Page, A. Peck, S. Perkins, M. Piercy, F. Qin, J. Quackenbush, S. Ranby, T. Reif, S. Rozen, C. Sanders, X. She, J. Silva, D. K. Slonim, C. Soderlund, W.-L. Sun, P. Tabar, T. Thangarajah, N. Vega-Czarny, D. Vollrath, S. Voyticky, T. Wilmer, X. Wu, M. D. Adams, C. Auffray, N. A. R. Walter, R. Brandon, A. Dehejia, P. N. Goodfellow, R. Houlgatte, J. R. Hudson Jr., S. E. Ide, K. R. Iorio, W. Y. Lee, N. Seki, T. Nagase, K. Ishikawa, N. Nomura, C. Phillips, M. H. Polymeropoulos, M. Sandusky, K. Schmitt, R. Berry, K. Swanson, R. Torres, J. C. Venter, J. M. Sikela, J. S. Beckmann, J. Weissenbach, R. M. Myers, D. R. Cox, M. R. James, D. Bentley, P. Deloukas, E. S. Lander, T. J. Hudson

The human genome is thought to harbor 50,000 to 100,000 genes, of which about half have been sampled to date in the form of expressed sequence tags. An

Proceedings of the National Academy of Sciences, Vol 90, 11995-11999, Copyright © 1993 by National Academy of Sciences

ARTICLE

Number of CpG Islands and Genes in Human and Mouse

F Antequera and A Bird

Estimation of gene number in mammals is difficult due to the high proportion of noncoding DNA within the nucleus. In this study, we provide a direct measurement of the number of genes in human and mouse. We have taken advantage of the fact that many mammalian genes are associated with CpG islands whose distinctive properties allow their physical separation from bulk DNA. Our results suggest that there are s45,000 CpG islands per haploid genome in humans and 37,000 in the mouse. Sequence comparison confirms that about 20% of the human CpG islands are absent from the homologous mouse genes. Analysis of a selection of genes suggests that both human and mouse are losing CpG islands over evolutionary time due to de novo methylation in the germ line followed by CpG loss through mutation. This process appears to be more rapid in rodents. Combining the number of CpG islands with the proportion of island-associated genes, we estimate that the total number of genes per haploid genome is s0,000 in both organisms.

CORRESPONDENCE

Nature Genetics 8, 114 (1994) doi:10.1038/ng1094-114a

Predicting the total number of human genes

Francisco Antequera¹ & Adrian Bird¹

¹Institute of Cell and Molecular Biology, Darwin Building, University of Edinburgh, Scotland EH9 3JR, UK

NEWS AND VIEWS

Nature Genetics 7, 345 - 346 (1994) doi:10.1038/ng0794-345

How many genes in the human genome?

Chris Fields¹, Mark D. Adams¹, Owen White¹ & J. Craig Venter¹

¹The Institute for Genomic Research, 932 Clopper Road, Galthersburg, Maryland 20878, USA

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letter

Estimate of human gene number provided by genomewide analysis using *Tetraodon nigroviridis* DNA sequence

Hugues Roest Crollius, Olivier Jaillon, Alain Bernot, Corinne Dasilva, Laurence Bouneau, Cécile Fischer, Cécile Fizames, Patrick Wincker, Philippe Brottier, Francis Quétier, William Saurin & Jean Weissenbach

- 28,000 34,000
- Based on alignments to pufferfish (*Tetraodon nigroviridis*)

letter

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Analysis of expressed sequence tags indicates 35,000 human genes

Brent Ewing & Phil Green

 Based on expressed sequence tag (EST) alignments to human chromosome 22

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letter

Gene Index analysis of the human genome estimates approximately 120,000 genes

Feng Liang, Ingeborg Holt, Geo Pertea, Svetlana Karamycheva, Steven L. Salzberg & John Quackenbush

- Based on assemblies of ESTs
- Extrapolated to whole human genome

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corrections

Gene Index analysis estimates the human genome contains 120,000 genes

F. Liang et al.

Nature Genet. 25, 239-240 (2000).

- Correction to avoid over-counting immunoglobulin ESTs
- 81,000 genes, based on assemblies of ESTs
- 57,000 genes, extrapolating from Chr 21 and 22

The gene count guessing game

Science 19 May 2000: Vol. 288. no. 5469, pp. 1146 - 1147 DOI: 10.1126/science.288.5469.1146 < Prev | Table of Contents | Next >

NEWS OF THE WEEK

HUMAN GENOME PROJECT: And the Gene Number Is ...?

Elizabeth Pennisi

COLD SPRING HARBOR, NEW YORK--Even though a draft sequence of the human genome is nearing completion, biologists still don't know how many genes it contains. Indeed, the range of estimates seems to be growing rather than shrinking. The question lies at the core of our understanding of genetic complexity. If genomes are the books of life, then genes are the words that tell the story of each organism. Biologists have long assumed that microorganisms are short stories and complex organisms such as humans, great tomes.



Place your bet. Uncertainty over the number of human genes has sparked a debate--and a betting pool.

Human genome paper I: *Nature* 409(15 Feb 2001), 860-921

articles

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium*

* A partial list of authors appears on the opposite page. Affiliations are listed at the end of the paper.

- 30,000 40,000 genes
- Large degree of uncertainty about total
- Number of distinct transcripts and proteins even less certain

Human genome paper II: *Science* 291(16 Feb 2001), 1304-51

The Sequence of the Human Genome

J. Craig Venter,1+ Mark D. Adams,1 Eugene W. Myers,1 Peter W. Ll,1 Richard J. Mural,1 Granger G. Sutton,¹ Hamilton O. Smith,¹ Mark Yandell,¹ Cheryl A. Evans,¹ Robert A. Holt,¹ Jeannine D. Gocayne,1 Peter Amanatides,1 Richard M. Ballew,1 Daniel H. Huson,1 ennifer Russo Wortman,¹ Qing Zhang,¹ Chinnappa D. Kodira,¹ Xianggun H. Zheng,¹ Lin Chen,¹ Marian Skupski, 1 Gangadharan Subramanian, 1 Paul D. Thomas, 1 Jinghui Zhang, George L. Gabor Miklos,² Catherine Nelson,³ Samuel Broder,¹ Andrew G. Clark,⁴ Joe Nadeau,⁸ Victor A. McKusick," Norton Zinder," Arnold J. Levine," Richard J. Roberts," Mel Simon," Carolyn Siayman, 10 Michael Hunkapiller, 11 Randall Bolanos, 1 Arthur Delcher, 1 Ian Dew, 1 Daniel Fasulo, 1 Michael Flanigan,1 Liliana Florea,1 Aaron Halpern,1 Sridhar Hannenhalti,1 Saul Kravitz,1 Samuel Levy,1 Clark Mobarry,¹ Knut Reinert,¹ Karin Remington,¹ Jane Abu-Threideh,¹ Ellen Beasley,¹ Kendra Biddick,¹ Vivien Bonazzi,¹ Rhonda Brandon,¹ Michele Cargill,¹ Ishwar Chandramouliswaran,⁶ Rosane Charlab,¹ Kabir Chaturvedi,¹ Zuoming Deng,¹ Valentina Di Francesco,¹ Patrick Dunn,¹ Karen Eilbeck,¹ Carlos Evangelista, 3 Andrei E. Gabrielian, 7 Weiniu Gan, 7 Wangmao Ge, 7 Fangcheng Gong, 7 Zhiping Gu, 7 Ping Guan,¹ Thomas J. Heiman,¹ Maureen E. Higgins,¹ Rui-Ru Ji,¹ Zhaoxi Ke,¹ Karen A. Ketchum, Zhongwu Lai,¹ Yiding Lei,¹ Zhenya Li,¹ Jiayin Li,¹ Yong Liang,¹ Xiaoying Lin,¹ Fu Lu,¹ Gennady V. Merkolov,¹ Natalia Milshina,¹ Helen M. Moore,¹ Ashwinikumar X. Naik,¹ Vaibhav A. Narayan, 1 Beena Neelam, 1 Deborah Nusskern, 1 Douglas B. Rusch, 1 Steven Salzberg, 12 Wei Shao," Bixiong Shue,1 Jingtso Sun,1 Zhen Yuan Wang,1 Aihui Wang,2 Xin Wang,1 Jian Wang Ming-Hui Wei,1 Ron Wides,13 Chunlin Xiao,1 Chunhua Yan,1 Alison Yao,1 Jane Ye,1 Ming Zhan,1 Weiging Zhang," Hongyu Zhang," Qi Zhao, 1 Liansheng Zheng," Fei Zhong, " Wenyan Zhong," Shiaoping C. Zhu,¹ Shaying Zhao,¹² Dennis Gilbert,¹ Suzanna Baumhueter,¹ Gene Spier,¹ Christine Carter,¹ Anibal Cravchik,¹ Trevor Woodage,¹ Feroze Ali,¹ Huijin An,¹ Aderonke Awe,¹ Danita Baldwin,1 Holly Baden,1 Mary Barnstead,1 Ian Barrow,1 Karen Beeson,1 Dana Busam,1 Amy Carver,1 Angela Center,1 Ming Lai Cheng,1 Liz Curry,1 Steve Danaher,1 Lionel Davenport,1 Raymond Desilets," Susanne Dietz, 1 Kristina Dodson, 1 Lisa Doup, 1 Steven Ferriera, 1 Neha Garg, 1 Andres Gluecksmann,¹ Brit Hart,¹ Jason Haynes,¹ Charles Haynes,¹ Cheryl Heiner,¹ Suzanne Hladun,¹ Damon Hostin,1 Jarrett Houck,1 Timothy Howland,1 Chinyere Ibegwam,1 Jeffery Johnson,1 Francis Kalush,¹ Lesley Kline,¹ Shashi Koduru,¹ Amy Love,¹ Felecia Mann,¹ David May,¹ Steven McCawley, 1 Tina McIntosh, 1 by McMullen, 1 Mee Moy, 1 Linda Moy, 3 Brian Murphy, 1 Keith Nelson,1 Cynthia Pfannkoch,1 Eric Pratts,1 Vinita Puri,1 Hina Qureshi,1 Matthew Reardon,1 Robert Rodriguez,¹ Yu-Hui Rogers,¹ Deanna Romblad,¹ Bob Ruhfel,¹ Richard Scott,¹ Cynthia Sitter,¹ Michelle Smallwood,1 Erin Stewart,1 Renee Strong,1 Ellen Suh,1 Reginald Thomas,1 Ni Ni Tint,1 Sukyee Tse, 1 Claire Vech, 1 Gary Wang, 1 Jeremy Wetter, 1 Sherita Williams, 1 Monica Williams, 1 Sandra Windsor, 1 Emily Winn-Deen, 1 Keriellen Wolfe, 1 Jayshree Zaveri, 1 Karena Zaveri, 1 Josep F. Abril, 14 Roderic Guigo, 14 Michael J. Campbell, 1 Kimmen V. Sjolander, 1 Brian Karlak, 1 Anish Kejariwal, ¹ Huaiyu Mi, ³ Betty Lazareva, ³ Thomas Hatton, ¹ Apurva Narechania, ¹ Karen Diemer, ¹ Anushya Muruganujan,¹ Nan Guo,¹ Shinji Sato,¹ Vineet Bafna,¹ Sorin Istrail,¹ Ross Uppert,¹ Russell Schwartz,¹ Brian Walenz,¹ Shibu Yooseph,¹ David Allen,¹ Anand Basu,¹ James Baxendale,¹ Louis Blick,1 Marcelo Caminha,1 John Carnes-Stine,1 Parris Caulk,1 Yen-Hui Chiang,1 My Coyne,1 Carl Dahlke,1 Anne Deslattes Mays,1 Maria Dombroski,1 Michael Donnelly,1 Dale Ely,1 Shiva Espanham,1 Carl Fosler,1 Harold Gire,1 Stephen Glanowski,1 Kenneth Glasser,1 Anna Glodek,1 Mark Gorokhov,1 Ken Graham,¹ Barry Gropman,¹ Michael Harris,¹ Jeremy Heil,¹ Scott Henderson,¹ Jeffrey Hoover, Donald Jennings,1 Catherine Jordan,1 James Jordan,1 John Kasha,1 Leonid Kagan,1 Cheryl Kraft,1 Alexander Levitsky," Mark Lewis," Xlangjun Liu," John Lopez," Daniel Ma," William Majoros," Joe McDaniel,* Sean Murphy,* Matthew Newman,* Trung Nguyen,* Ngoc Nguyen,* Marc Nodell,* Sue Pan,¹ Jim Peck,¹ Marshall Peterson,¹ William Rowe,¹ Robert Sanders,¹ John Scott,¹ Michael Simpson,* Thomas Smith, 1 Arlan Sprague,1 Timothy Stockwell, 1 Russell Turner,1 Eli Venter,1 Mei Wang,¹ Meiyuan Wen,¹ David Wu,¹ Mitchell Wu,¹ Ashley Xia,¹ Ali Zandieh,¹ Xiaohong Zhu¹

- 26,588 genes
- 12,000 additional "likely" genes based on similarity to mouse or other evidence

Steven Salzberg,¹²

Human genome version 2.0: *Nature* 431 (21 October 2004)

Finishing the euchromatic sequence of the human genome

International Human Genome Sequencing Consortium*

* A list of authors and their affiliations appears in the Supplementary Information

The sequence of the human genome encodes the genetic instructions for human physiology, as well as rich information about human evolution. In 2001, the International Human Genome Sequencing Consortium reported a draft sequence of the euchromatic portion of the human genome. Since then, the international collaboration has worked to convert this draft into a genome sequence with high accuracy and nearly complete coverage. Here, we report the result of this finishing process. The current genome sequence (Build 35) contains 2.85 billion nucleotides interrupted by only 341 gaps. It covers \sim 99% of the euchromatic genome and is accurate to an error rate of \sim 1 event per 100,000 bases. Many of the remaining euchromatic gaps are associated with segmental duplications and will require focused work with new methods. The near-complete sequence, the first for a vertebrate, greatly improves the precision of biological analyses of the human genome including studies of gene number, birth and death. Notably, the human genome seems to encode only 20,000–25,000 protein-coding genes. The genome sequence reported here should serve as a firm foundation for biomedical research in the decades ahead.

• 20,000 - 25,000 genes

How do we find genes?

- Ab initio gene finding
- Expressed sequence tags (ESTs)
- Full-length cDNA sequencing
- Alignment of protein sequences to genomic DNA
- Combining all the evidence together

Training a Gene Finder





ACCURACY

	Nuc Sens	Nuc Spec	Nuc Accur	Exon Sens	Exon Spec	Exact Genes	Size of test set
D.rerio	93%	78%	86%	77%	69%	24%	549 genes
C.elegans	96%	95%	96%	82%	81%	42%	1886 genes
Arabidopsis	97%	99%	98%	84%	89%	60%	809 genes
Cryptococcus	96%	99%	98%	86%	88%	53%	350 genes
Coccidioides	99%	99%	99%	84%	86%	60%	503 genes
Brugia	93%	98%	95%	78%	83%	25%	477 genes

GlimmerHMM has been trained on several species including Arabidopsis thaliana, Coccidioides species, Cryptococcus neoformans, and Brugia malayi. New: trainings for C. elegans and Danio rerio (zebrafish) are now available!

GlimmerHMM has been recently trained on human. The table below presents its performance compared to Genscan on 963 human RefSeq genes selected randomly from all 24 chromosomes, non-overlapping with the training set. The test set contains 1000 bp of untranslated sequence on either side (5' or 3') of the coding portion of each gene.

	Nuc Sens	Nuc Spec	Nuc Acc	Exon Sens	Exon Spec	Exon Acc	Exact Genes
GlimmerHMM	86%	72%	79%	72%	62%	67%	17%
Genscan	86%	68%	77%	69%	60%	65%	13%

How do we find genes?

- Ab initio gene finding
- Expressed sequence tags (ESTs)
- Full-length cDNA sequencing
- Alignment of protein sequences to genomic DNA
- Combining all the evidence together

Putting it all together manually

• View *ab initio* predictions and sequence alignments within a genome editor:



Apollo



Annotation Station



Artemis

Putting it all together automatically with JIGSAW



Evaluating methods on 1% of the human genome: ENCODE

GENES IN ACTION

VIEWPOINT

The ENCODE (ENCyclopedia Of DNA Elements) Project

The ENCODE Project Consortium*†

The ENCyclopedia Of DNA Elements (ENCODE) Project aims to identify all functional elements in the human genome sequence. The pilot phase of the Project is focused on a specified 30 megabases (\sim 1%) of the human genome sequence and is organized as an international consortium of computational and laboratory-based scientists working to develop and apply high-throughput approaches for detecting all sequence elements that confer biological function. The results of this pilot phase will guide future efforts to analyze the entire human genome.

approaches, such as cDNA-cloning efforts (4, 5) and chip-based transcriptome analyses (6, 7), have revealed the existence of many transcribed sequences of unknown function. As a reflection of this complexity, about 5% of the human genome is evolutionarily conserved with respect to rodent genomic

With the complete human genome sequence now in hand (1-3), we face the enormous challenge of interpreting it and learning how to use that information to understand the biology of human health and disease. The ENCyclopedia Of DNA Elements (ENCODE) Project is predicated on the belief that a comprehensive catalog of the structural and functional components encoded in the human genome sequence will be critical for understanding human biology well enough to address those fundamental aims of biomedical research. Such a complete catalog, or "parts list," would include protein-coding

Science 306 (2004), 636-640.

ENCODE Gene finding ASsessment Project (EGASP)



The GencodeDB Genome Browser. Fig. 2 from Guigo et al., Genome Biology 2006, 7(Suppl 1):S2.



EGASP results: Exon prediction accuracy among 28 different methods

From Guigo et al., Genome Biology 2006, 7(Suppl 1):S2

EGASP results: overall gene accuracy

Genefinding Method	Sens	<u>Spec</u>
AUGUSTUS-any	47.9	35.5
FGENESH++	69.9	42.0
JIGSAW	72.6	65.9
PAIRAGON-any	69.5	61.3
AUGUSTUS-abinit	24.3	17.2
GENEMARK.hmm-A	15.2	3.2
GENEMARK.hmm-B	16.8	7.9
GENEZILLA	19.5	8.8
ACEVIEW	63.5	48.6
AUGUSTUS-EST	47.6	37.0
ENSEMBL	71.6	67.3
EXOGEAN	63.1	80.8
EXONHUNTER	21.9	6.3
PAIRAGON+NSCAN_EST	69.5	61.7
AUGUSTUS-dual	26.0	18.6
DOGFISH	10.8	14.6
MARS	33.4	24.9
NSCAN	35.4	36.7
SAGA	4.3	3.4
GENSCAN	15.5	10.1
KNOWNgene	77.0	72.7
TWINSCAN	22.3	20.2

So, where are we now?

Ensembl genes www.ensembl.org



- 21,774 "Known genes"
- 1,036 Novel genes
- 3,994 RNA genes
- 69,185 Genscan gene predictions

Let's not forget pseudogenes

- 27,130 pseudogenes
- www.pseudogene.org

Current NCBI gene counts www.ncbi.nih.gov

- Entrez Gene:
 - 38,621 genes
 - But this includes pseudogenes
- RefSeq:
 - 28,961 genes
 - 31,784 transcripts

S NCRI	Consensus CDS protein set	CCDS Database			
NCDI	EDL + NO	CCDS Database CCDS			
PubMed	Ebi • NG	BLAST OMIM			
Search All	v for				
Clear					
Clear					
CCDS	Initial statistics for Homo	sapiens for build 35.1			
Home					
FTP Process	Current statistics for Homo sapiens for build 35.1				
Statistics	Initial statistics for Mus m	ausculus for build 36.1			
A HALLAND	initial statistics for wids in	lusculus for build 50.1			
EBI	Current statistics for Mus musculus for build 36.1				
NCBI					
UCSC					
	Initial statistics for <i>Homo sapiens</i> for build 35.1				
Contact Us	an of March 2, 2005				
GenComp eMail	as of March 2, 2005	Feb. 26, 2007			
Genome Displays	CCDS Totals				
E Ensembl	Category	Count 18 200			
U Genome Browser	CCDS IDs	14,795			
V VEGA	Gene IDs	13,142 16,008			
	Sequence IDs	31,724 51,388			
Related Resources					
Entrez Gene	Sequence IDs by Organi	ization			
RefSeq	NCBI RefSeq	15,496 19,360			
UniGene	EBI,WTSI Records	16,228 32,028			
	ConoID				
	Genes with >1 CCDS	1 500			
	ID	1,205 1,708			
	1 Contract				

Lesson: Science isn't decided by voting So, we still don't have a gene count

...and for many genes, we aren't yet sure of their exon-intron structure...

...and there are > 1000 other genomes already complete or under way...

...so we aren't giving up!

Thanks...







Mihaela Pertea



Jonathan Allen (LLNL)



Brian Haas (Broad Institute)



Bill Majoros (Duke Univ.)



Art Delcher

NLM

United States National Library of Medicine National Institutes of Health

Evaluating Gene Predictions



Fig. 3 from Guigo et al., Genome Biology 2006, 7(Suppl 1):S2.