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Physical Mapping of Human DNA: An Overview of the DOE Program

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The Highest Resolution Physical Map Is the DNA Sequence

Introduction

Just as a map of the earth's surface details geographical landmarks and distances, a map of DNA provides similar information about the human genome. For the DNA map, however, the landmarks are not cities, but genes or restriction enzyme recognition sites, and the distances between landmarks are not in miles, but in numbers of base pairs (bp). DNA maps can have either a genetic or a physical basis and offer various degrees of resolution. Generally, a strategy for physical mapping is chosen that is consistent with the interests of the scientists and with the laboratory's general programmatic effort.

There are two primary types of physical maps: a *macrorestriction map* and an *ordered-clone map*. The highest resolution physical map is, of course, the DNA sequence itself – the ultimate goal of the human genome effort. Given the present state of sequencing technologies, it is neither economically feasible nor technologically appropriate to begin a large-scale project now to sequence the entire genome of man. Rather, the construction of physical maps, as a first priority, will facilitate future sequencing efforts. Knowledge of the physical map and its correlation to the genetic map will guide the scientific community in assigning priorities to regions of the genome to be sequenced. A physical map of DNA clones can provide the raw material for sequencing.

"A physical map of DNA clones can provide the raw material for sequencing."

Macrorestriction Maps

A macrorestriction map is a linearly ordered set of large fragments of DNA representing a chromosome region or, potentially, an entire human chromosome. The

In This Issue. . .

Physical Mapping (continued)

Macrorestriction Map Serves as Global Map of Fragments Spanning Large Regions of DNA

fragments are derived by cutting very high molecular weight DNA with restriction enzymes whose recognition sites are of low occurrence in the genome. Since the dinucleotide sequence CpG is estimated to be underrepresented in the human genome by about fivefold, restriction enzymes that contain such dinucleotides as part of their recognition site will cut the DNA infrequently. Typical of the restriction enzymes in this category are *Not* I (GCGGCCGC) and *Mlu* I (ACGCGT), which theoretically cut, on the average, every million or 300,000 bp, respectively.

To obtain such large fragments of DNA, shearing must be minimized during the DNA isolation process. First, the cells are embedded in agar blocks, and then the DNA fragments are electrophoresed out of the agar blocks and separated using an alternating-field electrophoresis system. Depending on the conditions of electrophoresis, fragments ranging in size from about 50 kilobases (Kbp) to 10 megabases (Mbp) can be seen either by staining the gel with ethidium bromide or by performing Southern blots and identifying specific fragments by hybridization to radiolabeled probes.

Not I Sites Often Cluster in Islands Located Adjacent To Gene Sequences

Even with the rare-cutting restriction enzymes, the human genome contains too many fragments to separate on a single gel lane. Thus for most mapping studies, hybrid cells containing a single human chromosome are used, and the human chromosome-specific fragments are identified by Southern hybridization using total human repetitive DNA, human Alu-sequence probes, or human unique sequence probes (Fig. 1).

A macrorestriction map, developed by using rare-cutting enzymes, provides information at a level of organization between the intact chromosome and cloned fragments of DNA. It serves as a global map of fragments spanning large regions of DNA. More detailed maps will then be related to the global macrorestriction map.

Obtaining relative order of the restriction fragments is also possible. For example, techniques have been developed to construct probes that contain rare-cutting restriction sites and their flanking DNA. These are called *linking probes* because they uniquely identify adjacent restriction fragments (Fig. 1). If DNA is digested with *Not* I and hybridized to a *Not* I linking probe, the two adjacent fragments will be identified on the gel. A collection of such linking probes would then allow one to order the *Not* I restriction fragments for the chromosome or region of interest.

Another interesting application exploiting the rare-cutting restriction sites is to identify DNA polymorphisms. Since these sites are rich in CpG, which are also targets for methylation, the methylation sensitivity of many of the rare-cutters prevents them from cleaving the methylated site. In comparing two sources of DNA, differences in restriction fragment patterns might, therefore, identify patterns of DNA methylation. Finally, it has been shown that *Not* I sites

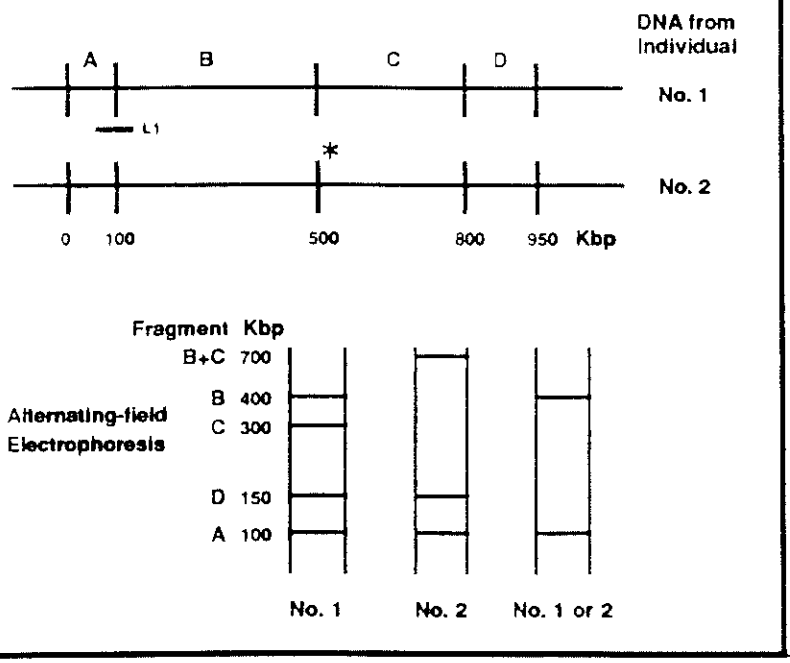

Fig. 1. Constructing a macrorestriction map using large-fragment electrophoresis. *Top:* A segment of DNA from two individuals, No. 1 and No. 2. Vertical lines represent the location of *Not* I restriction sites, and the total length in Kbp is shown below. One of the *Not* I sites in individual No. 2 is methylated, as indicated by the asterisk. A *Not* I linking probe is also shown as L1. *Bottom:* Three gel lanes with *Not* I fragments separated by alternating-field electrophoresis. The left lane shows the fragments derived from individual No. 1. The middle lane is DNA from individual No. 2. Note the absence of two smaller fragments and the presence of a new larger fragment due to the methylated site. The right lane indicates the two fragments that hybridize to the *Not* I linking probe in both individuals.

Physical Mapping (continued)

will often cluster in islands that are located adjacent to gene sequences. This information can be used to signal gene locations in a segment of DNA.

The Human Genome Program in the Department of Energy (DOE) Office of Health and Environmental Research supports a number of projects that develop or make use of macrorestriction maps of DNA. To highlight the various strategies and techniques, a few of these research activities are delineated here.

At Columbia University, Cassandra Smith and Charles Cantor have been constructing maps of the entire human chromosome 21 and the Huntington's region on the short arm of chromosome 4. Using somatic cell hybrids containing human chromosome 21 and several rare-cutting restriction enzymes, as well as human repetitive, unique sequence, and linking probes, Smith and Cantor estimate that about 40 Mbp of the chromosome can be recognized by the rare-cutter fragments on a gel. The researchers are in the process of determining the order of the fragments.

Thomas Caskey and his colleagues at the Baylor College of Medicine have established a macrorestriction map spanning a 7-Mbp region on the human X chromosome containing the G6PD gene cluster.

A group under the direction of Michael McClelland at the University of Chicago is exploiting the methylation sensitivity of some of the rare-cutting enzymes. By using methylation-sensitive restriction enzymes together with modification methyltransferases, McClelland's group is able to control partial digest reactions. Moreover, by first methylating adenine with a methyltransferase and then using methylation-dependent rare-cutters, very large DNA fragments (3–5 Mbp) are produced in some genomes. In conjunction with Carol Westbrook, also at Chicago, the researchers plan to apply these techniques to create a macrorestriction map for a region of chromosome 5.

While the rare-cutter restriction map is extremely useful, it does not provide cloned DNA for further analysis. Complementary methods which make use of cloned DNA are necessary.

Ordered Clone Maps

An ordered clone map is a collection of cloned DNA fragments arranged in the same linear position that they would have along the native chromosome. The clones are generally derived from primary arrayed libraries, and each clone is maintained either in the well of a microtiter tray or in an individual tube. The DNA may be cloned in any one of the vector systems [e.g., yeast artificial chromosomes (YACs), cosmids, phage, or even plasmids]. The larger the cloned insert, the lower the map resolution; but, fewer clones must be analyzed to construct the map. Three of the DOE-supported efforts to create clone maps rely primarily on cosmids which contain about 40 Kbp of insert. However, large, human DNA inserts of more than 100 Kbp are being cloned by YAC cloning vectors now being developed at Los Alamos National Laboratory (LANL), Lawrence Berkeley Laboratory (LBL), and Lawrence Livermore National Laboratory (LLNL), in collaboration with university-based investigators.

Clones from multiple libraries, and possibly from multiple vector systems, are believed to be necessary to complete a map of the entire chromosome. At least two factors contribute to this assumption:

Macrorestriction Map Strategies and Techniques Are Highlighted

The Genetic Map: A Tool To Locate Relative Positions of Genes

The genetic map is an important and complementary tool in human genome research. It can be used to locate the relative positions of genes, to assist in the study of the heritability of genetic diseases, and, ultimately, to validate the physical map. A genetic map of DNA provides information on the relative location of genes or gene markers (e.g., restriction fragment length polymorphisms, RFLPs). The distances on the genetic map are measured in centimorgans (cM), a measure of the recombination frequency between loci. The greater the distance between two loci, the greater the probability a meiotic recombination event will occur. While the genetic map does provide relative order for the loci on a chromosome, the frequency of recombination is not an accurate measure of physical distance. It is now well documented that some regions of DNA recombine much more frequently than others; therefore, the recombination frequency will either over- or underestimate the true distance in base pairs. The resolution of the genetic map is dependent upon the number of meiotic recombination events observed between loci as detected by RFLPs in two- or three-generation families with a large number of sibs. ◇

Physical Mapping (continued)

1. The inability to clone certain DNA sequences in some vector systems results in nonrepresentative libraries.
2. The nonuniform growth of individual clones leads to sample bias during clone selection.

Approaches for ordering cloned DNA fragments are based either on fingerprinting the cloned inserts or on searching for homology between the clones based upon DNA hybridization. The two methods, illustrated in Figs. 2 and 3, are discussed briefly below.

Clone Fingerprinting Methods

Clone fingerprinting methods attempt to identify a unique signature or fingerprint for each insert. Fingerprints are generally produced by digesting randomly selected clones from a primary arrayed library with one or more restriction enzymes (Fig. 2). After the restriction fragments are separated on a gel (either polyacrylamide or agarose), their fragment lengths are determined. Generating a complete restriction map of the clone is not necessary, but a sufficient number of fragments which uniquely identify that clone must be created. The fragment lengths from each clone are then compared to every other clone, and a statistical analysis is applied to determine if any clones possess a significant number of fragments of the same length. Inserts that have some portion of their fingerprint in common are then said to *overlap*. To a first approximation, the more similar the fingerprint, the greater the overlap. Thus, the ability to detect overlap between clones by this method depends on such factors as:

- the number of fragments that constitute the fingerprint,
- the ability to resolve fragments of different lengths on a gel, and
- the power of the statistical tests.

If other information can be added to fragment length, such as DNA sequence information for one or more fragments in the fingerprint, the ability to discriminate *true* from *false* overlap increases.

Random clone fingerprinting techniques generally require the analysis of a fivefold to tenfold clone redundancy in order to achieve about a 70–80% coverage of the chromosome or region of interest. These techniques can be laborious and computationally intensive. On the other hand, because the tasks are repetitive, they are amenable to automation, which decreases the likelihood of human error and optimizes both throughput and reproducibility. Also, because fingerprinting methods alone are unlikely to yield a complete map, they must be complemented with other procedures (e.g., macrorestriction mapping or hybridization techniques) to achieve map closure.

Both LLNL and LANL have adopted clone fingerprinting methods as a first step toward the construction of ordered clone maps. At Livermore, a method has been developed to label the ends of all restriction fragments from a single cosmid with a fluorescent dye. The fluorophor-labeled fragments are separated in a high-resolution denaturing polyacrylamide gel using a modified, commercially available, automated DNA sequencer. Afterwards, fragments in the gel are detected as they migrate past a laser beam. In the present configuration, four fluorochromes can be used; thus, up to three cosmids plus a labeled size standard can be analyzed per gel lane (i.e., 96 cosmids per single gel run). Data collection is automatic, and analysis of the fingerprints is performed off-line with minimal operator intervention. Using this technique, the LLNL group has assembled a set of overlapping cosmids that span a 600-Kbp region of

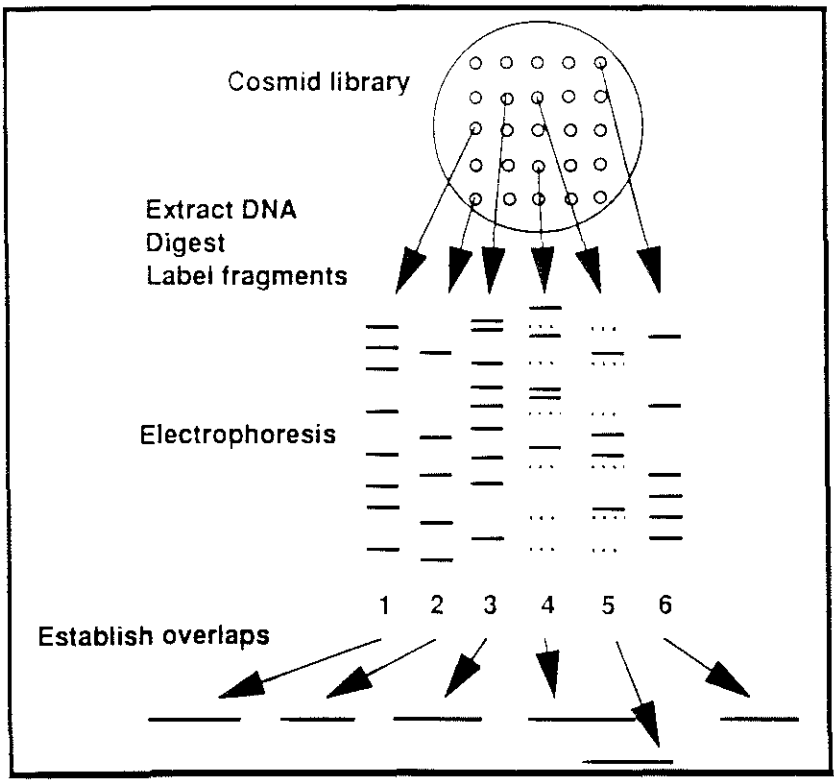

Fig. 2. Electrophoresis-based method for clone fingerprinting. Restriction fragments for each clone are separated; for each pairwise combination of clones, the fragment lengths (and perhaps additional information) are compared to determine statistical similarity (i.e., the extent of clone overlap). The dotted lines for clones 4 and 5 represent shared fragments.

Physical Mapping (continued)

chromosome 14, analyzed over 1500 cosmids (approximately onefold cosmid coverage) from chromosome 19, and assembled over 100 contigs (sets of overlapping clones).

The group at LANL has developed another approach to clone ordering. Their approach exploits certain repetitive DNA sequences and uses them as nucleation sites for mapping random clones. In this approach, individual clones are fingerprinted by a combination of restriction enzyme digestion and DNA hybridization. Restriction fragments from each clone are separated on agarose gels; their lengths are determined by comparison to size standards. Additional information is obtained for each clone by hybridizing the fragments on the gel with a repetitive DNA probe, which identifies a sequence that is present, on average, in 30–50% of the cosmids. The pattern of repetitive DNA hybridization, along with restriction mapping information, is acquired by image capture and analyzed by conventional algorithms. The result is a highly informative fingerprint of each clone. Using this approach, the Los Alamos group has processed about 2000 cosmids (approximately onefold coverage) and has assembled them into over 100 contigs.

Constructing overlapping sets of clones by detecting DNA sequence homology is a powerful and rapid strategy for clone mapping. Recently, the feasibility of this strategy has been successfully tested on a small region of human chromosome 11 by Glen Evans at the Salk Institute. This approach makes use of cosmid vectors that have bacteriophage T3 and T7 promoters flanking the cloned insert. The promoters are used to produce an RNA transcript that can be used as a hybridization probe. The probes from the ends of the cosmid insert are then hybridized to an array of cosmids representative of a chromosome region. A cosmid in the array that hybridizes to the probe should share sequence homology with the cosmid from which the probe was derived and, hence, should overlap it. The hybridization must be conducted at adequate stringency, and repeated sequences that would potentially cause false positive hybridizations must be blocked by prehybridizing the probe transcript with pooled, repetitive DNA.

In practice, a cosmid library is constructed for a chromosome region of interest and organized in replicate rectangular filter arrays so that each cosmid has a fixed coordinate. An oversimplification of the strategy is shown in Fig. 3. After DNA mixtures of cosmid clones are pooled from one row (e.g., row 2, replica 1 in Fig. 3), transcripts are prepared from the pooled mix, and the probes are hybridized to one of the filters. Similarly, pooled probes from one column (e.g., column B, replica 2 in Fig. 3) are hybridized to the replicate. Positive hybridization signals are obtained with all cosmids from the row and column from which the probes were derived. In addition, as shown in the figure, another cosmid at array location D3 hybridized to both the row and column probes. The only common cosmid from the row and column probes is located at the intersection of the row and column pools (i.e., B2). Thus the cosmid at B2 must overlap with the cosmid at D3. This technique, which has the sensitivity to detect very small regions of overlap, has been applied by Evans to a 32 × 36 array

(continued on page 6)

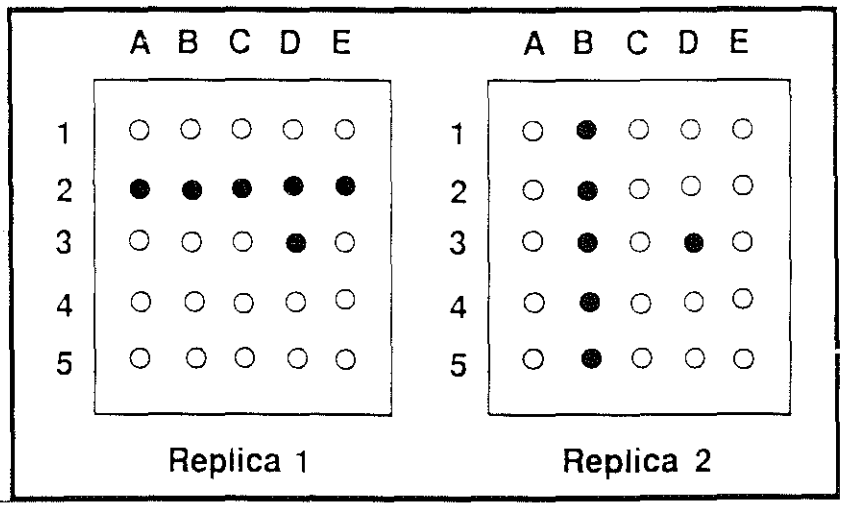

Fig. 3. Hybridization-based method for determining clone overlap. Cosmids are arrayed in a matrix on filters and replicated. Two sets of pooled hybridization probes are prepared; one from the members of a row and the other from the members of a column (e.g., row 2 and column B). The pooled probes are hybridized (one set to each filter). One of the two cosmids in an overlap is the common cosmid at the intersection of the row and column probes (i.e., B2). The other overlapping cosmid is the positive clone in both arrays other than the row and column positives (i.e., D3).

Physical Mapping Strategies (continued)

with about 1000 cosmids from chromosome 11q13-qter. After 68 (row plus column) hybridizations, he was able to establish over 300 contigs ranging in size from 2 to 27 cosmids. Whether or not this method can be scaled to deal with larger genomic regions remains to be determined.

In this overview, some of the physical mapping strategies currently being supported by the DOE Human Genome Program have been summarized. The laboratory techniques used to implement these strategies are state of the art and continually being improved. There is no *one optimal strategy* for physical mapping. In fact, new methods probably will be developed that replace or refine all of the present approaches. As those who are involved in physical mapping quickly realize, none of these techniques alone will produce a complete map. The final map likely will be derived from a combination of the strategies discussed above, as well as others yet to come. ◇

"The final map likely will be derived from a combination of the strategies discussed above, as well as others yet to come."

Journal Articles To Feature Human Genome Programs

The October issue of *Genomics* will feature three articles relevant to the human genome programs of the U.S. Department of Energy (DOE) and the National Institutes of Health (NIH). Benjamin J. Barnhart (DOE) and Elke Jordan (NIH) will author descriptions of their respective programs, and Robert M. Cook-Deegan will contribute an historical perspective pertaining to the 1984 Alta Conference, where the international human genome effort began. ◇

Meetings and Workshops

Update:

Contractor/Grantee Workshop
Nov. 3–4, 1989
Santa Fe,
New Mexico

Workshop Planned for DOE Human Genome Program Contractors and Grantees

The U.S. Department of Energy (DOE) Contractor-Grantee Workshop will be held Nov. 3–4, 1989, at the Santa Fe Hilton in Santa Fe, New Mexico. The DOE Human Genome Steering Committee (HGSC) is organizing the workshop for the Office of Health and Environmental Research (OHER) to:

- foster exchange of information among participants in the DOE Human Genome Program,
- evaluate individual projects, and
- evaluate the overall DOE program.

Approximately 150 representatives from all grantee and contractor projects are expected to attend this workshop and to present project highlights by giving either oral or poster presentations. The workshop, which the Santa Fe Institute is helping to coordinate, will be a major discussion topic at the HGSC meeting that follows on Nov. 5. Contractors/grantees should contact Sylvia J. Spengler, Lawrence Berkeley Laboratory, for technical information. For administrative information such as hotel arrangements, they should contact Andi Sutherland of the Santa Fe Institute. ◇

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Administrative Information Contact:

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(505) 984-8800

Cold Spring Harbor Laboratory

Genome Mapping and Sequencing Meeting

Cold Spring Harbor Laboratory Meeting
April 26–30, 1989
Cold Spring Harbor, New York

Technical advances presented at the 1989 Cold Spring Harbor Laboratory meeting on Genome Mapping and Sequencing led participants to conclude that a complete ordered clone map for all human chromosomes will be forthcoming in the next few years. Acknowledged by the genome research community as the world's foremost assembly for dissemination of the latest genome mapping and sequencing research results, the Cold Spring Harbor meeting was a forum for presenting key research efforts and latest advances in this rapidly developing area.

Credit should be given to the organizers – Charles Cantor, Maynard Olson, and Richard Roberts – for the excellent program and well-run meeting, which was held April 26–30, 1989, in Cold Spring Harbor, New York.

The following are some impressions and highlights of the meeting, particularly from the perspective of the U.S. Department of Energy (DOE) Human Genome Program.

Gene libraries. The gene libraries being produced at Los Alamos National Laboratory (LANL) and Lawrence Livermore National Laboratory (LLNL) are being used by numerous researchers around the world and are making a significant impact on human genome research.

Telomere studies. As an indication of the speed at which the field is moving – last year one paper on the DNA sequence of the human telomere was presented (Bob Moyzis, LANL). This year an entire and interesting session was devoted to telomere studies.

Physical mapping. DOE-sponsored comprehensive physical mapping of human chromosomes is progressing well and represents the major share of the world's total efforts in this research area. One very promising new approach is the mapping by nucleation at repetitive sequences being done at LANL. Other important contributions included: improved in-situ hybridization technology, radiation hybrid mapping, utilization of the polymerase chain reaction, and yeast artificial chromosome (YAC) cloning.

Techniques and Resources Needed To Complete Ordered Clone Map

Techniques and Resources Needed To Complete an Ordered Clone Map Of The Human Genome Are Now Considered To Be In Hand, or at Least Within Reach

The techniques and resources needed to complete an ordered clone map of the human genome are now considered to be in hand, or at least within reach, and they include:

- the various fingerprinting approaches (e.g., mapping by size, restriction patterns, and nucleation at repetitive sequences);
- radiation hybrid mapping;
- hybridization patterns of oligonucleotide probes to large insert libraries;
- YAC or other very large insert vector systems;
- DNA libraries;
- purification of large DNA fragments;
- improved in-situ hybridization;
- rare-cutter linking clones;
- polymerase chain reaction; and
- the growing number of markers on the genetic map. ◇

Submitted by:
David A. Smith, Acting Director
Health Effects Research Division
Office of Health and Environmental Research
U.S. Department of Energy

Meetings and Workshops

Tenth International Human Gene Mapping Workshop
June 11–17, 1989
Yale University
New Haven,
Connecticut

Tenth International Human Gene Mapping Workshop

Over 2000 New Gene Loci and DNA Markers Added to HGML Database

The goal of the International Human Gene Mapping workshops, initiated in 1973 at Yale University, has been to provide a forum in which members of the human genetics research community can summarize the current state of the human gene map, which is maintained by the Howard Hughes Medical Institute's Human Gene Mapping Library (HGML) at Yale University. At the recent Tenth International Workshop (HGM10) in New Haven, Connecticut, approximately 600 scientists from 28 countries presented new mapping data for comparison and integration with existing maps to produce a current and accurate account of the human gene map.

During this week-long workshop (June 11–17, 1989) 29 committees met representing the following areas:

- individual chromosomes or chromosome pairs,
- the mitochondrial genome,
- nomenclature,
- neoplasia and rearrangements,
- cytogenetic markers,
- linkage and gene order,
- clinical inherited disorders and chromosomal deletion syndromes,
- DNA segments,
- informatics, and
- comparative mapping.

The committees gathered and evaluated new information for recently discovered gene loci, anonymous DNA fragments, and disease phenotypes – all of which are linked to a specific chromosome or locus. The new mapping data were integrated into the existing HGML database, a publicly accessible information resource, by using an on-line database system. Chairpersons of these committees reviewed and entered over 2000 new gene loci or DNA markers.

In addition to new entries, the workshop committees revised existing HGML entries of genes or DNA markers to reflect recent literature reports or presentations at HGM10. Prior to this meeting, the human gene map database had contained approximately 1800 gene loci and an equal number of anonymous DNA segments regionally localized or genetically linked to specific loci.

The HGML database of genes, DNA markers, and disease loci has approximately doubled since the last workshop (HGM9) held in 1987. The rate of data generation is expected to increase significantly as a consequence of the rapid emergence of both new knowledge and technologies in all areas of gene mapping. These areas include the following levels:

- cytological (e.g., in-situ hybridization and somatic cell hybrids),
- linkage (e.g., genetic linkage and radiation fragmentation hybrids), and
- physical mapping (e.g., pulsed-field gel maps and ordered cosmid, yeast artificial chromosome, or phage clone contig maps).

Exponential growth of human genome mapping information can be projected to continue at an accelerated pace as data are generated by existing mapping projects (funded by the U.S. Department of Energy, the National Institutes of Health, and other agencies and organizations) and as new projects are initiated.

In addition to the large contributions made to the human gene map at the meeting, another significant outcome was the movement toward integration of mapping information arising from the various levels of mapping resolution (e.g., cytological, linkage, and physical). Initiatives taken by several of the workshop chromosome committees to use an integrated format to represent multilevel mapping information indicated that major challenges are ahead for the informatics and genetics/genomics communities.

The proceedings and outcome of the HGM10 Workshop reflect a high level of enthusiasm and commitment in the human genetics community to the task of coordination and cooperation in moving toward an integrated analysis of the human genome. Reports on the proceedings of the HGM10 workshop, including committee summaries and updated maps and

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Chromosome maps and database information are available on request from:

Human Gene Mapping Library
25 Science Park
New Haven,
CT 06511

E-mail:GENESIC @YALEVM.BITNET

Phone:
(203) 786-5515

Chromosome 16 Workshop

Composite Maps Completed with Data from Several Laboratories

Scientists engaged in mapping human chromosome 16 met at the Yale University School of Medicine in New Haven, Connecticut, on June 8–9, 1989. The workshop was jointly supported by the U.S. Department of Energy and the National Institutes of Health and was convened by Stephen Reeders. The purpose of the meeting was to integrate information from multiple levels of gene mapping and to construct an up-to-date series of chromosome maps based on various genetic and physical mapping techniques. The 28 attendees represented 16 laboratories from 7 countries.

Chromosome 16 mapping is proceeding at several levels. The following topics, listed here after their respective researcher(s), were included in workshop discussions:

Tim Keith *(Collaborative Research, Waltham, Massachusetts)* and **Cecile Julier** *(CEPH - Centre d'Étude du Polymorphisme Humain, Paris, France):* Genetic maps constructed using reference families – Over 80 polymorphic loci have been mapped using reference families, with loci spaced at average intervals of ~2.5 cM (in the 195-cM+ female map).

Peter O'Connell *(HHMI - Howard Hughes Medical Institute, Salt Lake City, Utah):* Automated techniques – O'Connell uses automated techniques to overcome sample handling errors, which are a major source of mistakes in genotypes and a major limitation in high-resolution genetic mapping.

Peter Lichter *(Yale University, New Haven,* Conn.): High-resolution in-situ hybridization – New techniques for multicolor fluorescence in-situ hybridization on metaphase chromosomes developed in David Ward's laboratory are capable of resolving probes spaced approximately 1 Mb apart.

Grant Sutherland *(Adelaide Children's Hospital, North Adelaide, Australia):* Chromosome break points – A series of chromosome break points effectively divides the chromosome into 25 intervals for rapid physical mapping of new clones.

Giovanni Romeo *(Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, Genoa, Italy):* Radiation fragmentation – The use of radiation fragmentation hybrids adds significantly more power to physical mapping by hybrid analysis.

Paul Liu *(The University of Texas MD Anderson Cancer Center, Houston, Tex.):* Selectable markers for enhanced mapping resolution – CHO hybrids retaining human ERCC4 activity have allowed Liu to create a high-resolution map of the 16p13 region.

Anne-Marie Frischauf *(ICRF - Imperial Cancer Research Fund, London, U.K.):* Radiation fragmentation hybrids – Her efforts focus on selecting genes from a region by cloning *Not* I sites from radiation fragmentation hybrids.

Stephen Reeders, Gerry Gillespie, and Greg Germino *(Yale University, New Haven, Conn.),* **Peter Harris** *(John Radcliffe Hospital, Oxford, U.K.),* and **Martijn Breuning** *(State University of Leiden, Leiden, the Netherlands):* Pulsed-field-gel mapping – Their work includes macrorestriction mapping in the region of the polycystic kidney disease locus.

Michael Siciliano *(The University of Texas MD Anderson Cancer Center, Houston, Tex.):* Somatic cell hybrid – He employs a novel technique to isolate human expressed sequences from a somatic cell hybrid.

Ray Stallings *(Los Alamos National Laboratory, Los Alamos, N.M.):* Overlapping cosmids – He is attempting to construct an array of overlapping cosmids for chromosome 16 starting with flow-sorted chromosomes.

Tom Marr *(Los Alamos National Laboratory, Los Alamos, N.M.):* Difficulties of handling data from multiple levels of mapping – He is working to develop an automated system with algorithms for detecting cosmid overlaps.

Participants divided into groups to complete composite maps based on the data from several laboratories. ◇

Chromosome 16 Workshop
June 8–9, 1989
Yale University
School of Medicine
New Haven,
Connecticut

Chromosome 16 Workshop maps may be obtained from:

Dr. Stephen T. Reeders
Yale University
School of Medicine
Department of
Internal Medicine
Nephrology/
2073 LMP
333 Cedar Street
New Haven, CT
06510-0856

Some of the maps will also be published in the report of the Chromosome 16 Committee (co-chaired by Stephen Reeders and Ed Hildebrand, Los Alamos National Laboraory, Los Alamos, N.M.) of the Tenth International Human Gene Mapping Workshop.

Gene Mapping Workshop

tables describing gene loci and anonymous DNA segments, will be published in an upcoming issue of *Cytogenetics and Cell Genetics*. The chromosome maps are available on request from HGML, which also offers five interconnected databases for use by those interested in human genetics. *(See sidebar on page 8 for HGML address.)* ◇

Meetings and Workshops

Radiation Research Society Annual Meeting
March 19–23, 1989
Seattle, Washington

Radiation Research Society Annual Meeting

Human Genome Effort to Benefit Radiation Biology Research

Benjamin J. Barnhart, Manager of the Department of Energy (DOE) Human Genome Program, emphasized the significance of DOE's program to the radiation biology community during his address at the Radiation Research Society's annual meeting held March 19–23 in Seattle. In his overview of the program, Barnhart pointed out that the program has as its basis the mission that DOE and its predecessor agencies have maintained for over four decades: to investigate the potential health effects of energy-related radiations and chemicals and to use DOE's unique laboratory capabilities to benefit society.

Barnhart went on to report that with its aim of developing the resources and technologies needed to characterize the human genome at the molecular level, the program is already providing some state-of-the-art biological and computational resources and technologies that facilitate studies of the genetic effects of environmental insults.

Many scientific presentations at the meeting, according to Barnhart, described research that will benefit from results of the multidisciplinary study of the human genome. These benefits would include the availability of:

- fluorescent-tagged DNA probes for cytogenetic analyses,
- large stretches of DNA sequence information for use in investigating molecular changes resulting from radiation exposures at very low doses, and
- extensive sequence data for use in studies of genetic rearrangements in radiation-induced diseases and in the analysis of the expression of radiation-altered genes such as oncogenes and tumor suppressor genes. ◇

X-Ray Microimaging for the Life Sciences Workshop
May 24–26, 1989
Berkeley, California

X-Ray Microimaging for the Life Sciences Workshop

Cellular and Subcellular Level Imaging Thought Possible in Next Five Years

The X-Ray Microimaging for the Life Sciences Workshop brought together the physical and biological science communities to discuss research opportunities afforded by new high-intensity, high-brightness, coherent X-ray sources, as well as by both advanced synchrotron and X-ray lasers.

Held in Berkeley, California, on May 24–26, 1989, and sponsored by the U.S. Department of Energy through the Lawrence Berkeley Laboratory, the workshop was attended by over 100 scientists. Workshop emphasis was on direct imaging, at spatial resolutions not previously possible, of biological materials in their natural state.

In general, the conferees were optimistic that imaging native structures at the cellular and subcellular levels with X-rays at resolutions as good as 100 Å should become possible within the next 5 years. Although imaging at the molecular level (10 Å resolution) was considered to be further off, Charles Rhodes did present a scheme for imaging and sequencing DNA by Fourier transform X-ray holography. According to Rhodes, a feasibility study on this technique will start soon.

Included in the workshop were some 30 oral and poster presentations covering the characteristics of new light sources, X-ray microscopy, X-ray holography, and other direct X-ray imaging techniques. The limitations of these techniques, due to radiation damage to the biological samples, were considered. Researchers are seeking to recognize this damage and to prevent or limit it.

Completing the picture were discussions on state-of-the-art technologies in optical microscopy, electron microscopy, X-ray diffraction, and scanning tunneling microscopy. Three-dimensional microscopic photos were included in the presentations.

Two preconference tutorials on biological structure were special features of the workshop for participants who did not have a background in the life sciences; the early morning classes were well attended. ◇

Submitted by:
Gerald Goldstein, Acting Director
Physical and Technological Research Division
Office of Health and Environmental Research
U.S. Department of Energy

Workshop proceedings, consisting of extended abstracts, will be available later this year from:

National Technical Information Service
(703) 487-4600

Meetings and Workshops

American Electrophoresis Society 1989 Annual Meeting

Electrophoretic Techniques in Molecular Biology

American Electrophoresis Society Annual Meeting
July 6–11, 1989
Washington, D.C.

The 1989 Annual Meeting of the American Electrophoresis Society (AES) was held July 6–11 in Washington, D.C. The society, whose president is Carl Merrill of the National Institutes of Health, conducted its first annual meeting to facilitate communication among researchers who are using electrophoretic methods, theories, and applications. The program included a comparable number of presentations relating to both protein and DNA electrophoretic separations strategies and methodologies.

Some presentations relating to genome mapping and sequencing efforts are highlighted below:

Leroy Hood *(California Institute of Technology)* gave a comprehensive assessment of the technology developments needed to confront the challenges associated with genome mapping and sequencing. In a presentation that encompassed both protein and DNA separations/sequencing schemes, Hood offered that deciphering the encyclopedia of life—the genetic code—will increase understanding of the multiplicity of interrelated biochemical events that comprise normal life processes. Also discussed were direct applications of genome sequencing work to medical research of abnormal life processes, particularly in the area of preventive medicine.

Norman Anderson *(Large Scale Biology Corp.)* discussed the use of the protein gene product as a starting point to obtain the corresponding DNA and, ultimately, the DNA sequence; the potential problems of creating large-scale systems from smaller functional systems; the need for standardization of reagents; and the forces that influence funding agencies.

Charles Cantor *(Lawrence Berkeley Laboratory)* described some of the latest pulsed-field gel (PFG) electrophoresis developments in his laboratory. In a session devoted to PFG techniques, he said that because no one has made a detailed, quantitative description of the pulsed-field phenomenon, there are more opportunities for serendipitous discoveries. Since the publication of the PFG method in 1984 by Schwartz and Cantor (*Cell* **37**:67), many variations and improvements have been made both in their laboratories and by others. With the advent of large-size standards, the implementation of the Smith-Birnstiel restriction mapping approaches, and the use of telomeric probes, large regions of human chromosomes can now be mapped efficiently by using PFG techniques.

David Patterson *(Eleanor Roosevelt Institute for Cancer Research)* described his work on mapping chromosome 21. He uses a modification of the PFG technique to gain a better understanding of the role that chromosome 21 trisomy plays in Down's Syndrome.

To illustrate his method of constructing computer models that demonstrate how DNA molecules travel through agarose gels, **Steven Smith** *(University of Washington, Seattle)* showed a dramatic videotape of stained DNA fragments (50 Kbp–1 Mbp), which were undergoing agarose gel electrophoresis. The process was filmed through a fluorescence microscope aided by an image intensifier. Using these video tapes, Smith is better able to model and understand the movement (and hence separation) of DNA molecules in PFG electrophoresis systems. A listing of his Pascal program source code for modeling movement of DNA through gels and a copy of the video tape can be obtained from the University of Washington *(see sidebar for addresses)*.

Michael Harrington *(California Institute of Technology)* described improvements for implementation of the two-dimensional gel electrophoresis (2-DGE) technique both in the clinical laboratory and in the study of developmental biology systems. Starting with the 2-DGE analysis of gene products (proteins) allows identification of the genes that code for those proteins. Discussed by both Hood and Anderson earlier in the meeting, these genetics techniques are complementary to the direct mapping and DNA sequencing approach.

Terry Landers *(Life Technologies)* reviewed a variety of techniques for modifying nucleic acids by incorporating radioisotopes and non-isotopic reporter groups for detection.

Bruce Roe *(University of Oklahoma)* and **Richard Wilson** *(California Institute of Technology)* gave useful presentations concerning automated approaches to DNA sequencing. ◇

Submitted by: Betty K. Mansfield, HGMIS

Videotape Information:

Instructional Media Services
Attn.: Sandra Wells
University of Washington
Kane Hall, DG10
Seattle, WA 98195
(206) 543-9921
(Video tape price: $60)

Pascal Source Code Information:

Internet:
STEVE@SNERT.CPAC.WASHINGTON.EDU

Interactions

U.S. Human Genome Effort: DOE/NIH Interactions

Human Genome National Plan Due February 1990

The U.S. Department of Energy (DOE) Human Genome Program, which began in 1987 with the goal of developing the resources and technologies needed to characterize the entire human genome at the molecular level, is now one of the two major components of this nation's human genome effort.

To receive sustained Congressional support for this effort, DOE and the National Institutes of Health (NIH) will formulate a *national plan*. This plan, which Congress originally requested from James Wyngaarden as an NIH plan, has been expanded in scope and now includes the DOE program. Its due date is February 1990.

When program scope, funding, and strategies are considered, the initiatives of the two agencies are quite complementary, as described below.

NIH Office of Human Genome Research. In 1988 the NIH (U.S. Department of Health and Human Services) formally started a human genome effort. James D. Watson, Nobel Laureate, was appointed Associate Director of NIH for Human Genome Research. Elke Jordan was named Director of the Office of Human Genome Research. Effective FY 1990, this office will become the National Center for Human Genome Research. Broad in scope, the NIH effort differs from the DOE program in several ways, because its program:

- focuses on comparative genetic studies of human and model organisms,
- promotes both predoctoral and postdoctoral training programs,
- emphasizes the study of disease genes, and
- funds research largely through individual university-related research grants.

DOE Human Genome Program. In contrast, the majority of DOE's work is conducted at the department's multidisciplinary national laboratories, and the focus of DOE's research and development program is to:

- construct linearly ordered maps of chromosome-specific DNA clones (prepared by the National Laboratory Gene Library Project);
- improve significantly the efficiency of sequencing DNA; and
- upgrade the computer capabilities needed to organize, disseminate, and interpret the sequence of the human genome.

DOE/NIH Coordination Progressing

Coordination of planning and activities between these two federal agencies with formal genome programs is progressing; therefore, more effective management within and between the two programs should be facilitated.

DOE Steering Committee Attends NIH Program Advisory Committee for the Human Genome

The NIH Program Advisory Committee for the Human Genome held its second meeting in Bethesda, Maryland, June 19–20, 1989. Benjamin J. Barnhart, Manager of the DOE Human Genome Program, attended the meeting and gave a progress report on the DOE program. All five members of the DOE Human Genome Steering Committee (HGSC) actively participated in the meeting.

Points of discussion included:

- NIH center grant requests, which are due January 1990;
- how quickly and in what form mapping sequence data should become available;
- the need for nomenclature standards; and
- funding for instrumentation.

The next NIH Program Advisory Committee meeting will be held Dec. 4–5.

DOE Steering Committee Activities

DOE/NIH To Convene Joint Planning Committee To Develop National Plan

At the DOE HGSC meeting (April 18, 1989) in which members of other agencies, including NIH representatives Mark Guyer, Elke Jordan, and James Watson participated, DOE and NIH agreed to convene a

HGSC Meeting Reports can be obtained from the DOE Human Genome Management Information System at Oak Ridge National Laboratory. **(See order form on page 16.)**

joint planning committee to develop a national human genome plan with both an NIH and a DOE component. This committee will meet at the end of August and prepare a draft report, which will then be considered by the DOE Health and Environmental Research Advisory Committee (HERAC) and by the NIH Human Genome Advisory Committee.

A second working session of this joint planning committee will be held in early October 1989. Both DOE and NIH will approve any revised draft document before it is finalized; the plan will then be submitted to each agency's respective Congressional subcommittees as the aforementioned national plan.

Sample and Information Sharing

Subsequent to the April 18 HGSC meeting, some of the attendees met at Cold Spring Harbor to discuss issues of sharing samples and information. The committee concluded that these issues transcended individual national programs; it was announced that a subcommittee of the international Human Genome Organization (HUGO) will be creating guidelines this summer for distribution to individual national program leaders for comment.

DOE Computational Task Force and NIH Database Working Group

Just as DOE and NIH personnel are attending each other's major genome-related meetings – in accordance with the guidelines set forth in the October 1988 memorandum of understanding between the two agencies – there are also plans to merge the capabilities of the DOE Computational Task Force (see Human Genome Quarterly, Vol. 1, No. 1, page 5) with the NIH Database Working Group. Since data manipulation and management are global issues, this combined group of respected scientists may eventually become international in both membership and scope.

Other Issues Considered at HGSC Meetings

During the April 18 meeting in Bethesda and the July 18 meeting in Houston, other issues considered were:

- the policies for sharing individual clones, arrayed libraries, and master arrays; and
- national laboratory technology transfer issues.

The next HGSC meeting will be held Nov. 5 in Santa Fe, New Mexico. ◇

Instrumentation Concepts and Technologies Being Surveyed

Willana Consultants, Cheshire, U.K., is conducting an international survey of instrumentation technologies and methodologies being used for DNA (mapping and sequencing) of the human genome. Sponsored by the Human Genome Management Information System (HGMIS) at Oak Ridge National Laboratory, the survey will:

- provide an update on the concepts and technologies being employed in DNA sequencing and on their potential application and
- determine what innovative improvements and new concepts are needed to speed up and optimize the mapping and sequencing of complex genomes.

The results of the study will be published in the spring of 1990 as a technical report and will be available from the HGMIS.

Willana Consultants' William J. Martin and Paul F. G. Sims, who are conducting the survey, invite scientists and engineers to submit relevant material to them by September 30, 1989. Acknowledgement for information received and incorporated into the text will accompany the appropriate material in the report.

Concepts and technologies being surveyed for study include:

- innovative, high-risk concepts for DNA sequencing;
- prospects for linking various devices to form integrated systems;
- potential usefulness of existing systems not currently employed in extensive DNA sequencing;
- challenges associated with scale-up of existing systems;
- instrumental methods of biotechnology data-capture;
- biomaterial manipulation; and
- macromolecule separation science.

For more information, please contact: Betty Mansfield at HGMIS or Willana Consultants *(see sidebar at top of page)*. ◇

Please submit material for the technical report to:

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011-44-61-200-4226
FAX:
011-44-61-236-0409

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1989–1990	Calendar of Genome Events	
August	24–26	**Mouse Genome Workshop**; Oxford, U.K. [*Mary Lyon, 44-235-84393*]
	28–30	**NIH/DOE Planning Working Group**
September	10–15	**198th American Chemical Society (ACS) National Meeting** (Human Genome Computing Sessions, Wednesday, Sept. 13); Miami Beach, FL [*ACS, (202) 872-4396*]
October	2–4	**Human Genome I: An International Conference on the Status and Future of Human Genome Research**; Town & Country Hotel, San Diego, CA; co-chairmen: Daniel E. Koshland, Jr., and Charles R. Cantor [*Scherago Assoc., Inc., (212) 730-1050*]
	2–4	**NIH/DOE Planning Working Group**
	6	**Recombinant DNA Advisory Committee**; Bethesda, MD
	24–26	**Genome Sequencing Conference**; Wolf Trap Conference Center, Reston, VA co-chairmen: J. Craig Venter and C. Thomas Caskey [*Craig Venter, FAX : (301) 480-8588*]
	25–27	**The Fourth San Diego Conference: Nucleic Acid Applications**; Hyatt Islandia on Mission Bay (San Diego), CA [*American Association for Clinical Chemistry (AACC), (619) 943-1541*]
November	3–4	**DOE Workshop for Contractors and Grantees in the DOE Human Genome Program**; Santa Fe, NM
	5	**DOE Human Genome Steering Committee**; Santa Fe, NM
	9–10	**Nature's 12th International Conference: Gene Manipulation in Biology and Human Disease**; Copley Plaza Hotel, Boston, MA [*Diane Berger, (212) 477-9600*]
	12–15	**American Society of Human Genetics (ASHG) Annual Meeting**; Baltimore, MD [*Jean Francese, (301) 571-1825*]
December	4–5	**NIH Program Advisory Committee on the Human Genome**; Bethesda, MD
	12–14	**DOE/NIH Large Insert Cloning Workshop**; Houston, TX
	14–15	**DOE/NIH X-Chromosome Workshop**; Houston, TX
January	22–28	**UCLA Symposium: Manipulating the Mammalian Genome**; organizers: Alan Bernstein, C. Thomas Caskey, and A. Dusty Miller; abstract deadline: September 13, 1989; Tamarron, CO [*UCLA Symposia, (213) 207-5042*]
March	20	**DOE Human Genome Steering Committee**, Pasadena, CA
April	10–13	**The First International Conference on Electrophoresis, Supercomputing and the Human Genome**; Florida State Conference Center, Tallahassee, FL; organizer: Hwa A. Lim [*Supercomputer Computations Research Institute, (904) 644-4518/3490*]
June	18–19	**NIH Program Advisory Committee on the Human Genome**; Bethesda, MD
	19–20	**DOE Human Genome Steering Committee**; Bethesda, MD

Publications: Historical Bibliography of the U.S. Human Genome Program

Technologies for Detecting Heritable Mutations in Human Beings
U.S. Congress, Office of Technology Assessment, OTA-H-298, U.S. Government Printing Office, Washington, D.C., September 1986.(NTIS: PB87140158)
- National Technical Information Service
  5285 Port Royal Road
  Springfield, VA 22161
  (703) 487-4600
  Price: $21.95 (U.S.)

Sequencing the Human Genome, Summary Report of the Santa Fe Workshop, March 3–4, 1986*
U.S. Department of Energy, Office of Health and Environmental Research, Los Alamos National Laboratory, Los Alamos, N.M., 1986.

Report on the Human Genome Initiative for the Office of Health and Environmental Research* ("HERAC Report")
Subcommittee on the Human Genome, Health and Environmental Research Advisory Committee (prepared for the Office of Health and Environmental Research, Office of Energy Research, U.S. Department of Energy), DOE, Germantown, Md., April 1987.

The Human Genome, Proceedings of the 54th meeting of the Advisory Committee to the Director, Oct. 16–17, 1986
National Institutes of Health, Bethesda, Md. 1987.
- Dr. Jay Moskowitz
  National Institutes of Health
  Building 1, Room 103
  Bethesda, MD 20892

Biotechnology and the Human Genome: Innovations and Impact
A. D. Woodhead, B. J. Barnhart, and K. Vivirito, eds., ISBN: 0-306-42990-X, Basic Life Sciences Series, Vol. 46 (based on the Science Writers Workshop held September 14–16, 1987, at Brookhaven National Laboratory) Plenum Press, New York, N.Y., 1988.
- Plenum Publishing Corporation
  233 Spring Street
  New York, NY 10013
  (212) 620-8047

Mapping and Sequencing the Human Genome
National Research Council of the National Academy of Sciences, ISBN: 0-309-03840-5, National Academy Press, Washington, D.C., 1988.
- National Academy Press
  2101 Constitution Avenue, NW
  Washington, DC 20418
  (202) 334-2000
  Price: $14.00 (U.S.), $18.00 (Can.)

Mapping Our Genes-The Genome Projects: How Big? How Fast?
U. S. Congress, Office of Technology Assessment, OTA-BA-373, U. S. Government Printing Office, Washington, D.C., April 1988.
- Superintendent of Documents
  U.S. Government Printing Office
  Washington, DC 20402-9325
  (202) 783-3238
  (GPO stock number 052-003-01106-9)
  Price: $10.00 (U.S.)

Report to the Director, National Institutes of Health
The Ad Hoc Program Advisory Committee on Complex Genomes, National Institutes of Health, Bethesda, Md., 1988.
- Ms. Anne Thomas, Director
  Division of Public Information
  National Institutes of Health
  Building 1, Room 340
  Bethesda, MD 20892

The Human Genome Initiative of the U.S. Department of Energy*
Office of Energy Research, Office of Health and Environmental Research, Department of Energy, DOE, Germantown, Md., September 1988.

DOE Human Genome Steering Committee Meeting Reports*

Minutes of the first meeting of the NIH Program Advisory Committee on the Human Genome (Jan. 3–4, 1989)
- Ms. Anne Thomas, Director
  Division of Public Information
  National Institutes of Health
  Building 1, Room 340
  Bethesda, MD 20892 ◇

*Available from HGMIS. See Subscription/Document Request on page 16.

Human Genome quarterly

This newsletter is intended to facilitate communication among genome researchers and to inform persons interested in genome research. Suggestions and contributions are invited.

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Acronym Index

Acronyms Found in the Human Genome Vernacular

Acronyms listed were chosen because they were either used in the text or relevant to the human genome research community. Listed in parentheses after an organization is the branch of government or the organization to which it is responsible.

*Denotes organizations that are within the U.S. Department of Energy.

†Denotes organizations that are within the U.S. Department of Health and Human Services.

2-DGE	Two-dimensional gel electrophoresis
AACC	American Association for Clinical Chemistry
ACS	American Chemical Society
AES	American Electrophoresis Society
ANL*	Argonne National Laboratory
ASHG	American Society of Human Genetics
BNL*	Brookhaven National Laboratory
CEPH	Centre d'Étude du Polymorphisme Humain
DHHS	U.S. Department of Health and Human Services
DOE	U.S. Department of Energy
HERAC*	Health and Environmental Research Advisory Committee
HGCTF*	Human Genome Computational Task Force
HGMIS*	Human Genome Management Information System (ORNL)
HGML	Human Gene Mapping Library (HHMI)
HGSC*	Human Genome Steering Committee
HHMI	Howard Hughes Medical Institute
HUGO	Human Genome Organization [international]
ICRF	Imperial Cancer Research Fund
LANL*	Los Alamos National Laboratory, Los Alamos, N.M.
LBL*	Lawrence Berkeley Laboratory, Berkeley, Calif.
LLNL*	Lawrence Livermore National Laboratory, Livermore, Calif.
NCHGR†	National Center for Human Genome Research (NIH) [FY 90]
NIH†	National Institutes of Health
NRC	National Research Council of the National Academy of Sciences
OER*	Office of Energy Research
OHGR†	Office of Human Genome Research (NIH)
OHER*	Office of Health and Environmental Research (OER)
ORNL*	Oak Ridge National Laboratory, Oak Ridge, Tenn.
OTA	Office of Technology Assessment (U.S. Congress)
PFG	Pulsed-field gel [electrophoresis]
RFLP	Restriction fragment length polymorphism
YAC	Yeast artificial chromosome