Exploiting the Genome

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In 1997, JASON conducted a DOE-sponsored study of the human genome project with special emphasis on the areas of technology, quality assurance and quality control, and informatics. The Report of that study (JASON Report #JSR-97-315) is available from the JASON Program Office and on the internet at http://www.ornl.gov/hgmis/publicat/miscpubs/jason/.

The present study has two aims: first, to update the 1997 Report in light of recent developments in genome sequencing technology, and second, to consider possible roles for the DOE in the ``post-genomic" era, following acquisition of the complete human genome sequence.

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1 INTRODUCTION

The human genome project is one of the most significant activities in contemporary science. It has had a profound impact on evolutionary biology, the molecular sciences, and biomedicine, and its importance will increase as the project approaches completion. The stated goal of the project is to obtain the complete sequence of the human genome by the year 2005. The genome contains approximately 3.3 Gb (billion base pairs). As of September 7, 1998, 0.20 Gb (6.1%) had been sequenced and deposited in the sequence database. The current pace of the worldwide sequencing effort is about 0.1 Gb per year. Roughly half of that effort is supported by the US Government, at an anticipated cost of $2.5 billion over the lifetime of the project. Approximately one-third of the US effort is supported by the Department of Energy. The DOE program includes the Joint Genome Institute (JGI), which is a collaborative enterprise involving the Lawrence Berkeley, Lawrence Livermore, and Los Alamos National Laboratories.

In 1997, JASON conducted a DOE-sponsored study of the human genome project, with special emphasis on the areas of technology, quality assurance and quality control, and informatics. The Report of that study (JASON Report JSR-97-315) is available from the JASON Program Office and on the internet at http://www.ornl.gov/hgmis/publicat/miscpubs/ jason/. A brief synopsis of the Report was published earlier this year in *Science* (Koonin, S.E. **279**, 36–37, 1998). The present study has two aims: first, to update the 1997 Report in light of recent developments in genome sequencing technology, and second, to consider possible roles for the DOE in the "post-genomic" era, following acquisition of the complete human genome sequence. The phrase "exploiting the genome" refers to various scientific and technical approaches to mining the burgeoning database of genome sequence information in order to understand the functional consequences of that information. This area of research often is referred to as "functional genomics".

2 TECHNOLOGY

The 1997 JASON Report made the following specific recommendations:

Technology

1. The DOE should play a leading role in technology development.
2. Improvements should be sought in Sanger dideoxy sequencing technology.
3. Funding should be increased for alternative sequencing technologies not based on gel electrophoresis.
4. The DOE sequencing centers should maintain flexibility with regard to incorporating new technologies.

Quality Assurance and Quality Control

5. QA/QC considerations should be made integral to the human genome project.
6. QA/QC issues should be treated quantitatively.
7. Standardized QA/QC protocols should be implemented across all sequencing centers.

Informatics

8. Let the "customers" determine what informatics tools should be made available.
9. Encourage standardization of data formats and provide translators that converge on those common formats.

10. Maintain flexibility with regard to database structure and query operations.

A major recommendation of the 1997 JASON Report concerned the need for greater attention and increased funding in the area of technology development. Some concern was expressed as to whether the existing sequencing technology would be sufficient to complete the human genome project by 2005. Even if that goal could be met, clear benefit would be derived from more robust and higher-throughput DNA sequencing methods.

In April, 1998 the DOE Office of Biological and Environmental Research issued a call for proposals in the area of genome instrumentation research (Program Notice 98-16, available at http://www.er.doe.gov/production/grants/fr98_16.html). That Notice solicited new applications involving "substantive improvements to current systems and novel and creative new strategies" for genome instrumentation. A total of $2 million will be available under this Program for awards to be made in FY 1999. This is a positive step and precisely the type of activity that is needed for the DOE to maintain its leadership role in technology development. However, the amount of funding is only about 2% of the total annual DOE budget for genome sequencing and may not be sufficient to develop and critically evaluate new technologies. It may be appropriate to increase funding for this Program in FY 2000, depending on the scope and quality of the proposals received in response to Program Notice 98-16.

The most dramatic development in human genome sequencing over the past year was the announcement on May 10, 1998 by The Institute for Genomic Research (TIGR) and Perkin-Elmer (PE) of their plan to form a joint venture to determine the complete human sequence by the year 2001. This would involve the formation of a new company, later named Celera Genomics, to be headed by TIGR President J. Craig Venter. The key to this private initiative is a set of enhanced DNA sequencing technologies centered on the new PE Applied Biosystems "ABI PRISM 3700" automated DNA sequencer. The new instrument employs capillary gel (versus traditional slab gel) electrophoresis technology and is designed to perform 960 sequence reads per day.

The instrument utilizes robotic liquid transfer and gel loading to reduce operator time to only 15 minutes per day. In contrast, the current-generation ABI PRISM 377 instrument performs 144 sequence reads and requires 8 hours of operator time per day. The PRISM 3700 will not be available to the general scientific community until Summer, 1999 and is projected to cost $300,000, about three times the cost of a PRISM 377, but is expected to be more economical to operate because of lower labor costs and reduced consumption of reagents.

Other companies and research organizations have developed DNA sequencers based on capillary gel electrophoresis technology. Molecular Dynamics offers the "MegaBACE 1000", which has been commercially available for more than a year. Like the PRISM 3700, this instrument performs ~1,000 sequence reads per day and utilizes automated sample injection to reduce operator time. SpectruMedix markets the "SCE9600", a capillary gel electrophoresis DNA sequencer that incorporates technologies developed at the Ames Research Laboratories through funding from the DOE. Beckman Coulter sells the "CEQ 2000" DNA sequencer, which utilizes many of the innovations in capillary gel electrophoresis that first were developed in their analytical capillary electrophoresis instruments.

There are technological enhancements associated with the new capillary gel electrophoresis instruments, some that can be applied to existing slab-gel electrophoresis DNA sequencers and others that will carry forward to the microchannel-based devices that are expected to succeed capillary gel electrophoresis sequencers. These enhancements include:

- **Improved dye-labeled terminators**. Dideoxynucleotides bearing an attached fluorescent dye are used to indicate which of the four nucleotides occurs at a particular read length. Improved dye systems, such as the PE "Big-Dye" dideoxynucleotides, employ fluorescence resonance energy transfer to couple a common energy donor to four different energy acceptors, each having a distinct wavelength. This provides equivalent light intensity for each of the four nucleotides, thus improving the quality of the sequence reads.

- **Engineered thermostable polymerases**. A DNA polymerase, preferably one that operates at high temperature, is used to extend a DNA primer in the presence of dideoxynucleotides. Naturally-occurring DNA polymerases are not optimized for the incorporation of dye-labeled dideoxynucleotides. However, variant forms of these enzymes have been developed that contain specific amino acid substitutions that improve the enzyme's ability to accept the bulky substrates.

- **Automated gel loading**. Robotic liquid transfer and gel loading increases sequencing throughput while reducing operator time. This technology is useful now and will be essential for future generations of ultra-high-throughput automated DNA sequencers.

- **Fixed-position CCD**. Rather than scanning across a slab gel while tracking the boundary between gel lanes, the PRISM 3700 and SCE9600 instruments utilize a fixed-position 2-dimensional CCD array that monitors a bundle of discrete capillaries. The CCD array scans in the spectral dimension, allowing the operator to choose the wavelengths to be monitored.

None of the new capillary gel electrophoresis instruments involves a departure from Sanger dideoxy sequencing methods. However, all provide a substantial increase in DNA sequencing throughput compared to current-generation instruments, especially when used in conjunction with the technological enhancements described above. This higher throughput allows one to consider alternative sequencing strategies that would otherwise be impractical, for example, the whole-genome shotgun sequencing strategy first proposed by Weber and Meyers (*Genome Res.* **7**, 401–409, 1997) and now being adopted by Celera.

The term "shotgun" refers to the many fragments of DNA that result when the input DNA is broken down into smaller pieces that are picked at random. These fragments are cloned, amplified, isolated individually, and further amplified to provide material for sequencing. The aim is to generate enough clones to furnish a statistical representation of the entire

input DNA, embodied within a set of overlapping clones whose sequences can be assembled computationally. All genome sequencing strategies make use of shotgun cloning at some point. The standard approach within the human genome project has been to divide each chromosome into a complete set of either large-sized (80–300 kb) or medium-sized (35–45 kb) segments of DNA, which are then used as the input for shotgun cloning and sequencing. This approach is referred to as "directed sequencing". In contrast, Celera's strategy is to use the entire human genome as the input for shotgun cloning.

Celera proposes to employ 230 of the ABI PRISM 3700 instruments to perform ~200,000 sequence reads per day, each consisting of >500 base pairs of raw sequence data. Most of these sequences will be derived from the ends of small (~2 kb) inserts obtained by random digestion and shotgun cloning of total human genomic DNA (for more information, see Venter *et al.*, *Science* **280**, 1540–1542, 1998). A smaller number of sequences will be obtained from the ends of larger (~10 kb) random inserts, and a still smaller number from the ends of very large (~150 kb) inserts within a library of bacterial artificial chromosomes (BACs). Together, these will provide the raw data for assembly of the finished sequence. At a pace of 200,000 sequence reads per day, only about 350 sequencing days would be required to obtain 10-fold sequence coverage of the entire human genome. By relating the raw sequence data to physically mapped sequence tagged site (STS) and expressed sequence tag (EST) sequences, it should be possible to assemble the bulk of the human genome sequence.

Unlike the directed sequencing strategy being implemented in a distributed manner by the JGI and other large sequencing centers, Celera aims to assemble the complete human genome sequence directly from a total of ~70 million end sequences. There is lively debate in the genomics community regarding the feasibility of this whole-genome shotgun sequencing approach. An important concern is the extent to which the completed sequence will contain gaps, and therefore not be truly complete. Celera anticipates that their sequencing strategy will leave several thousand gaps, although this may be a significant underestimate. Who will close the remaining gaps? A related concern is the extent to which the final sequence will be misassembled due

to the incorrect arrangement of the component sequence segments. This will be an especially serious problem within regions of the genome that contain highly repetitive sequences. Celera's announcement also raises a crucial question of how members of the federally-supported genomics community should adjust their efforts in light of its new initiative.

The JGI has been quick to respond in a positive and pragmatic manner to the TIGR-PE announcement. The DOE had already helped to lay the groundwork for Celera's sequencing campaign by funding work at TIGR and the University of Washington to sequence the ends of inserts within a library of BAC clones. These data will provide critical signposts for fixing the location of the vast number of end sequences that will be obtained from the smaller (2 and 10 kb) inserts. The JGI has proposed to test the shotgun sequencing strategy at the level of individual BAC clones. Each BAC clone of 100–200 kb will be divided into a set of smaller (~3 kb) plasmid inserts and the ends of these inserts will be sequenced. About 750 plasmids will be end-sequenced to provide 5-fold sequence coverage of the parent BAC. The end sequences then will be assembled to obtain the complete BAC sequence. This strategy will yield sequence assemblies that are of sufficient size to encompass a typical gene and will do so more rapidly compared to the whole-genome shotgun sequencing approach.

The following recommendations are made with regard to genome sequencing technology:

1. *Capitalize on and complement Celera's efforts.* Capillary gel electrophoresis DNA sequencers, such as the ABI PRISM 3700, together with associated technological enhancements, are certain to provide a dramatic increase in DNA sequencing throughput. It is less clear how difficult it will be to assemble the complete genome sequence from the vast number of end sequences that will be generated. By supporting the end-sequencing of BAC clones, the DOE has already made an important contribution to the assembly process. These clones may also be useful in closing many of the gaps left by Celera's total shotgun sequencing approach. Other gaps, especially those in regions that are not represented within BAC clones, will require more labo-

rious methods to achieve closure. There will be little incentive for a private company to carry the gap-closing process through to the end, although most biologists believe that there will be significant value in closing the gaps. It may turn out, however, that the most intractable gaps are not worth closing, provided that the size and location of these gaps are known. In any case, the JGI should be prepared to close those gaps that are worth closing.

2. *Proceed with BAC shotgun sequencing.* The plan to test the large-scale shotgun sequencing strategy at the level of individual BAC clones is a good one. In the near term, this will assist in the development of new software tools for sequence assembly and will reveal special problems that pertain to the wholesale assembly of human genomic sequence. This activity offers the best opportunity for synergy between the public and private sequencing efforts. In the long term, shotgun sequencing of individual BAC clones is likely to be useful in closing gaps left by Celera's sequencing efforts and may be applicable to the sequencing of other eucaryotic genomes.

3. *Transition to capillary gel electrophoresis sequencing.* Celera will take delivery over the next eight months of 230 of the new PRISM 3700 sequencers. PE Applied Biosystems expects to make a small number of these instruments available to other large customers within the next year. At present, the JGI is largely committed to the current-generation instruments and is employing a sequencing strategy that would not benefit substantially from the new higher-throughput machines. This must change. Capillary gel electrophoresis provides a significant advance that will be followed by a series of incremental improvements over the next few years. Some of these technologies will be carried forward to the next generation of microchannel-based sequencing devices. The JGI should keep abreast of the state of the art in commercial DNA sequencing instrumentation by bringing a few of the commercially available capillary gel electrophoresis based sequencers in house at the earliest opportunity. This will allow first-hand evaluation of the performance characteristics of these instruments, possibly stimulate changes in the JGI sequencing strategy, and promote flexibility in considering the future operation of the sequencing facilities. As the JGI becomes more active in

the shotgun sequencing of BAC clones, the high-throughput instruments will become more necessary.

4. *Continue advanced technology development.* The recent call for proposals in the area of genome instrumentation research is commendable. It is clear that technology developments over the past year have dramatically changed the landscape of human genome sequencing. There appears to be no limit to the need for further increases in DNA sequencing capacity. The genomics community cannot depend on a single company or single type of DNA sequencing methodology to ensure ongoing technological advancement. Development of new methods of sequencing, not based on gel electrophoresis, should be supported. More so than other agencies, the DOE has the obligation and capability to foster long-term technology development in the area of DNA sequencing.

3 FUNCTIONAL GENOMICS

A year ago one looked expectantly toward the "human index sequence", the complete sequence of the human genome that would be available by 2005. Although there was uncertainty as to whether the task would be completed on time, surely it would be reached within a few years later. Now there is a possibility that the (nearly) complete sequence of the human genome will be in hand by 2001 or shortly thereafter. There is a plan afoot within the JGI and the genomics community to produce a "draft" sequence of the human genome by 2001, focusing initially on regions that are well mapped and likely to be of biological interest. The draft sequence would contain numerous sequence gaps but few physical gaps, and would serve as a platform to anchor the finished sequence. Pushing the notion of what might constitute a draft sequence, Incyte Pharmaceuticals recently announced a plan to map its existing database of EST sequences onto the physical map of the genome, declaring the result to be the first complete (though privately held) picture of the human genome. The question of what constitutes a "complete" genome sequence is open to scientific and philosophical debate. Regardless of one's opinion, it is clear that the post-genomic era is about to begin.

The ultimate goal of exploiting the genome is to understand the function of each of the roughly 100,000 human genes, both individually and collectively, and to understand how those functions differ in different cell states, cell types, individuals, and organisms. Functional genomics is most meaningful when viewed across the entire genome. This is because understanding the interactions among gene products, whether to form structures, execute regulatory functions, or catalyze chemical reactions, is at least as important as the functions of the gene products viewed individually. The function of a gene includes its physical locus within the genome, the primary RNA transcript that derives from the gene, the succession of RNAs along the processing pathway to a mature messenger RNA (mRNA), the protein product that results from translation of the mRNA, the succession of chemical and conformational changes that the protein undergoes, and the association of the

protein into large multiprotein "machines". It also is important to consider the substantial portion of the genome that does not encode a gene product, but contains sequences that play important regulatory roles in gene expression. Functional genomics ultimately involves understanding all of biology, a task that will keep scientists occupied indefinitely.

It is clear that priorities must be set in exploring the endless frontier of functional genomics. The JASON study does not address scientific priorities at the level of investigator-initiated research. These priorities are best established by members of the biological research community and honed by the peer-review process. Rather, the study considers the role that the DOE might play to facilitate research in functional genomics at both academic institutions and the national laboratories. The following recommendations are made with regard to DOE support of functional genomics:

1. *Provide a library of full-length cDNA clones.* A substantial database of human EST sequences exists in both the public and private domains. Most of these sequences are of partial-length complementary DNA (cDNA), typically derived from the 3′-terminal portion of an expressed mRNA. It would be highly beneficial to generate an inventory of full-length cDNA clones and make them available to the scientific community. These clones would be useful for many aspects of functional genomics research, including expression analysis, mapping of cDNAs onto genomic sequence, and the preparation of proteins for biophysical studies. By analogy to the DOE's highly successful operation of synchrotron facilities, the cDNA clones might be offered as a shared resource to be utilized by individual investigators for varied purposes.

2. *Limit funding of polymorphism analysis.* The sequence of the human genome is described not only by the "index sequence" but also by sequence polymorphisms that distinguish one individual from another. Single nucleotide polymorphisms (SNPs) occur at an overall frequency of about one per thousand nucleotides and at a much greater frequency within certain regions of the genome. The location, identity, and population distribution of these polymorphisms has considerable biological and biomedical significance. For example, SNPs might be used to stratify patient populations with respect

to the treatment of disease. Not surprisingly, there is tremendous commercial interest in recognizing important SNPs and securing the associated intellectual property rights. The academic research community also has a strong interest in SNPs, most of this work falling under the purview of the National Institutes of Health. This is a fertile area for research and it already is receiving ample attention from other agencies. DOE's special strengths lie in other areas and, in our view, DOE will have more impact by directing its limited resources to other areas of genome science.

3. *Move aggressively into comparative genomics.* Interpretation of the human genome sequence requires comparison with other genomes, especially those of closely-related species. Celera has announced its intention to sequence the genome of the fruit fly, as a "warm-up" project that will be completed by Spring, 1999. It appears likely that they will attempt to sequence the mouse genome as well. The DOE has long supported comparative studies in mouse and human genomics and maintains a valuable genetic resource within its Mouse Source program at Oak Ridge National Laboratory. The mouse genome will be especially useful in identifying genes and regulatory regions within the human genome. The DOE should assume a strong role in comparative genome sequencing. This is an excellent use for the sequencing capacity that will be available following completion of the human genome and is an activity more closely associated with studies of biological diversity than biomedical applications. The DOE already has established its place in comparative genomics by supporting the sequencing of archael and eubacterial genomes. It now is time to contemplate a broader sweep across the tree of life. In considering which genomes to sequence the DOE should consult with individuals who have expertise in both eucaryotic taxonomy and molecular aspects of gene expression (such individuals are rare). The goal would be to acquire genome sequences at well-chosen phylogenetic intervals, preferably examining pairs of closely-related organisms at each interval.

4. *Assume responsibility for database management.* The topic of sequence database management was discussed extensively in the 1997 JASON Report. The key recommendation in this area was that the DOE should promote community-wide standards for software operation and the quality

of the entered data. It was suggested that database operations be viewed as a service offered to the marketplace of potential users and that databases be made appropriate to the needs and level of computational sophistication of the users. Similar needs apply to functional genomics databases, although in this case the community is more fragmented. Functional genomics databases that are likely to have broad utility include listings of co-expressed genes referenced to cell type and cell state, indexes of protein-protein interactions, and a compendium of solved structural domains. As with DNA sequence databases, it will be essential to develop software tools that perform automated checks on the quality and completeness of the entered data. A modular approach is needed so that the authoring and publishing functions performed by individual investigators are separated from the cataloguing and data manipulation functions performed by database curators. This will allow investigators to focus on data acquisition in the face of changing research methods while curators focuses on data management in the face of changing computer technology.

5. *Foster research on genome-wide expression analysis.* The difference between functional genomics and traditional efforts in biochemistry and molecular biology is that the former is viewed from a genome-wide perspective. Functional genomics demands a high degree of parallelism in its analytical methods. Examples of these methods include sequence-comparison algorithms, DNA arrays, protein expression arrays, high-throughput functional assays, and targeted gene knockouts. The DOE can foster progress in functional genomics by supporting technology development, with emphasis on genome-wide technologies.

4 SUMMARY

Genomics research is a sure bet. This will be one of the most prolific areas of scientific investigation over the next two decades. The human index sequence is only the beginning; it will be followed by studies involving polymorphism analysis, comparative genomics, and functional genomics that will revolutionize scientific understanding of biological systems. The DOE is playing a prominent role in genomics research and is positioned to have a major impact in the post-genomics era. Among the general lessons that emerged from this and the previous JASON study of the human genome project are the following: 1) continue to support advanced technology development, especially as it addresses the unquenchable thirst for enhanced DNA sequencing throughput; 2) be nimble in adopting new technologies as they become available; 3) serve the community by providing software and molecular services that have broad application. With these guiding principles and the recognition that "big science" does not connote monolithic science, it promises to be an exciting road ahead.

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