INSTITUTE FOR THEOLOGICAL ENCOUNTER WITH SCIENCE AND TECHNOLOGY

BULLETIN

DATE: FALL, 1992 VOL: 23 NO. 4

Perhaps as our Vice-Director, Bob Bertram, says of himself, I am leaving my dotage and entering into my anecdotage.

I have been reading Francis Fukuyama's *The End of History and the Last Man*. I find myself saying that if what he calls history is what is ending, thank God. If history has to do with the planned and logical working out of a rational destiny, I think I'd like to stop the world and get off.

Perhaps it's age, perhaps something else, but more and more I find myself discontented with the rational approach to the world. I find this intense post-Enlightenment romance with *Reason* increasingly empty and increasingly boring. I find it in the kind of science that's being written about — not necessarily with the kind of science that's being done — as well as with a great deal of what passes for theology these days.

I don't want to project this on anyone beyond myself, but I'm fed up with theories, especially theories about theories — which is what I think Fukuyama is treating. Maybe it's simply tedium or boredom, but I long for more spontaneity in the perception of life and, most especially, in its living. God did not give us a world where everything would fall into recognizable patterns if only we could find the correct theory. He did not set up a world where reason was the dominant end and means. I believe in my heart that he set up a world open to my (and everyone else's) spontaneity, passion and love. I see more clearly and yearn for more deeply a world where beauty is at least as important as reason — and vastly more important than logical planning.

We talk about a world where we shall plan the direction of our future growth and, indeed, from time to time it seems as if we are working out ways of achieving that. Perhaps we should think about that and ask ourselves if we want to live in a neat planned world or one messy with surprises. I personally will opt for the surprising world over the planned one. I'd like a world where we make a spontaneous contribution to the growth of the Kingdom, even if it's no more than an unplanned moment of awe before the beauty of a flower or a sunset or a person. Or God.

Robert Brungs, S.J.

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ANNOUNCEMENTS

1. Plans for our 25th anniversary celebration/convention in Holyoke, Massachusetts are progressing well. We have been fortunate in recruiting three excellent speakers for this convention: Dr. John Staudenmaier, S.J. (History of Technology, Detroit-Mercy University) on beauty in technology; Mr. Leonard Buckley (foreman of designers, Bureau of Engraving and Printing) on beauty in art; Bishop John Sheets, S.J., (Auxiliary Bishop of Fort Wayne/South Bend) on Christian beauty. We are still searching for a speaker on beauty in science. The presently known details follow on page 3 of this issue of the Bulletin. We shall keep you informed as the rest of the program is developed. We want to thank Maxyne Schneider, SSJ for the great help she has been and will be in planning for this Convention.

2. The topic for the October 23-25, 1992 workshop was *The Human Genome Project.* We had two scientists to press the case *for* the human genome project, two scientists who urge caution, a theologian, and a computer scientist who discussed how the data can be handled. This workshop was held at Fordyce House in St. Louis, Missouri. We hope to have the Proceedings in the mail for our dues-paid members by late March or April, 1993.

3. The Proceedings of our October, 1991 *Seminar with Fr. Stanley Jaki, OSB* have been mailed to all dues-paid members. The Board of Directors in April, 1992 decided to print the *Membership Directory, 1993* in the same volume with the October Proceedings. The sole reason for this was financial. The double volume saved a couple of thousand dollars. The *Directory* contains the names of dues-paid members as of 7/31/92.

4. As noted in the Summer issue of the *Bulletin*, several ITEST members are writing chapters for a book on Faith and Science Issues (title open to suggestions) that we intend to complete in time for the Holyoke Convention. It will include chapters on the methods employed in the various sciences, philosophy and theology. It will also contain some historical material on the growth of science and on the theology-science conflict and three chapters (Protestant, Orthodox and Catholic) on the elements of the Christian faith. We wish to thank the authors both for their willingness to write these chapters and for sharing their wisdom with us.

5. The Board of Directors has commissioned the staff to explore the possibility of producing an hour long film (in two independent segments) on the beauty of creation. With the help of Mr. Richard Cusack of Chicago, we have prepared a concept paper and have begun the process of raising the money ($100,000 +) for this project. If any of you know of any foundation we might approach, please let us know.

5. We announced in the Summer *Bulletin* that the Board of Directors had approved a preliminary survey on the feasibility of launching a quarterly magazine (roughly along the lines of *Science*). We mentioned that we would be sending each of you (before the end of the year) a mock-up of an issue, with all the articles taken from the ITEST archives for your reactions and suggestions. Because of the amount of work associated with the Holyoke Convention and because of other initiatives, the Staff simply does not have time to carry out this mandate at present. Consequently, this project has been "put on the back-burner" until at least next year at this time.

6. One of the "other initiatives" that the Board has decided on is an updated re-publication of the *ITEST Summary* done by Peggy Keilholz in 1983. This will be a major task. We hope to have it finished in time for the 25th anniversary celebration, but please be patient with us.

7. We like to mention that we have fewer than 60 copies left of *The Vineyard: Scientists in the Church*. If you would like to order more copies (at $9.95) please do so soon. There are no present plans for a second edition.

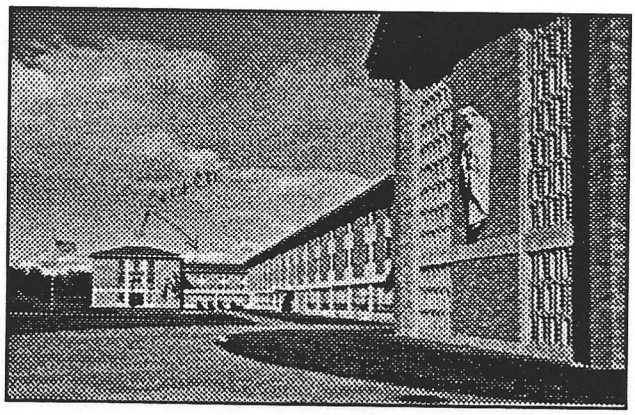
8. We have found over our almost-quarter-of-a-century experience that most of our long-term members have been recruited by other members. Please help us spread the Good News of Christ to our scientific-technological and theological colleagues. Let them know about us and let us know about them. In this way we will be better able to serve the Lord in this very crucial area of the church's life and growth.

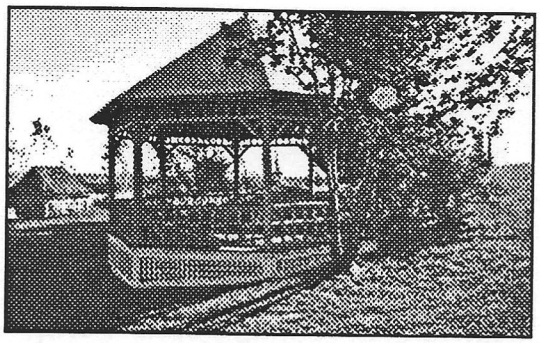
9. We wish you a very grace-filled Advent, a joyous and blessed Christmas season and a New Year in which we grow more deeply into the Lord.

ITEST

25TH ANNIVERSARY

CONVENTION

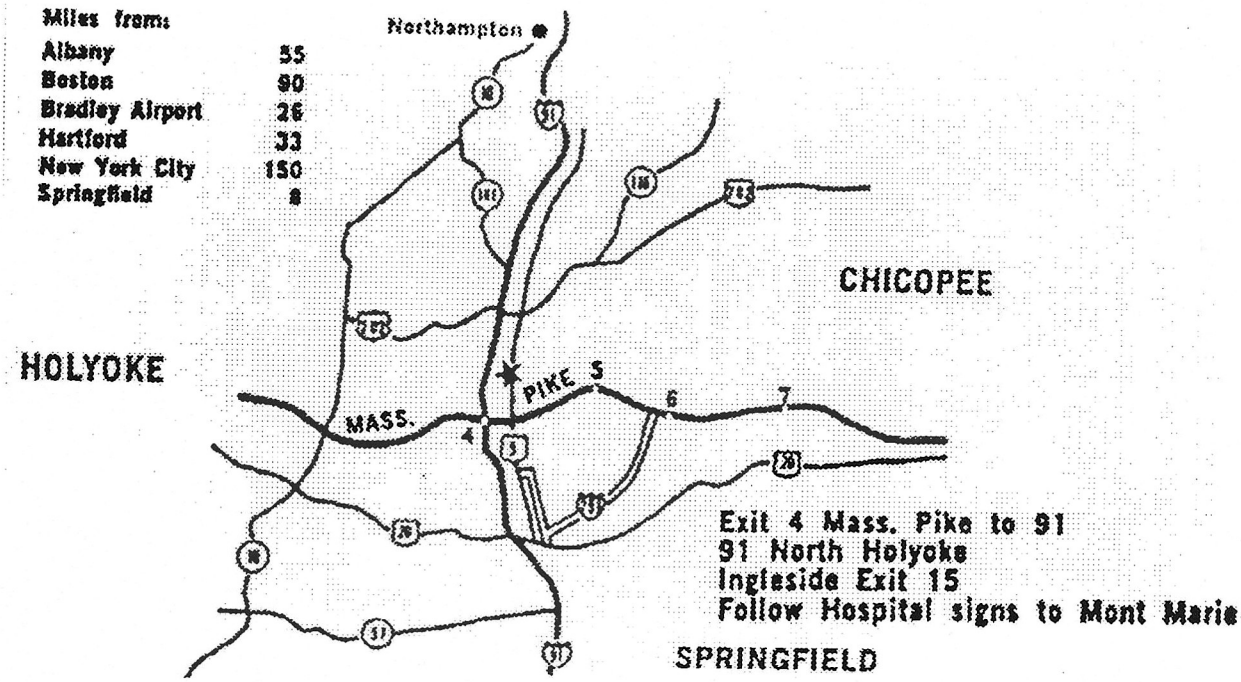




MONT MARIE

HOLYOKE, MASSACHUSETTS

AUGUST 1 - 6, 1993



COSTS

REGISTRATION and DEPOSIT: There will be a $25.00 registration and a $25.00 deposit fee, refundable before June 15, 1993. The deposit will be applied to the cost of the meeting.

SINGLE ROOM: **$27.00/day/person.** Spouses and children over 12; **$15.00/day/person**. Children under 12 are free. A few suites with double beds & bath are available on a first-come-first serve basis. These prices are final — they will not change.

MEALS:	Breakfast	(full)	$5.90
	Lunch		6.90
	Dinner		8.00
	Banquet (to be determined later)		

These prices are fixed.

CULTURAL/RECREATIONAL OPPORTUNITIES

Berkshire Mountains (1-2 hrs by car); Basketball Hall of Fame and cultural centers; a large amusement park in Holyoke area; children's museum, etc.; Old Sturbridge Village, a re-creation of mid-1800s New England village (>1 hr by car). There is a large swimming and ample grounds; picnics by the pool; large barbecue grill. Child care may be available, depending on interest. We suggest that the Convention be tied to a New England vacation.

A FORMAL INVITATION WILL BE SENT EARLY IN 1993.

SUNDAY August 1, 1993	MONDAY August 2	TUESDAY August 3	WEDNESDAY August 4	THURSDAY August 5	FRIDAY August 6
	9:00 - 10:15 am IV*	9:00 - 10:15 am II		9:00 - 10:15 am III	9:00 - 10:45 I-B
	10:15 - 10:45 am COFFEE BREAK	10:15 - 10:45 am COFFEE BREAK	FREE	10:15 - 10:45 am COFFEE BREAK	
	10:45 - 12:00 noon V	10:45 - 12:00 noon VI		10:45 - 12:00 noon I-A	10:45-11:30 VII
	LUNCH	LUNCH	LUNCH	LUNCH	DEPART
ARRIVE	FREE	FREE	FREE	FREE	
5:30 pm DINNER	5:30 pm DINNER	5:30 pm DINNER	5:15 WORSHIP	5:30 pm DINNER	
SOCIAL	7:30 LECTURE John Staudenmaier, SJ *Technology and Beauty*	7:30 pm LECTURE Mr. Leonard Buckley *The Artist and Beauty*	6:00 pm BANQUET	7:30 pm LECTURE M.R. John Sheets, SJ *The Christian Idea of Beauty*	
	SOCIALIZING	SOCIALIZING		SOCIALIZING	

* See next page for topic key.

KEY FOR MORNING SESSIONS

25TH ANNIVERSARY CONVENTION, HOLYOKE, MA

AUGUST 1 - 6, 1993

I-A	Perspectives on Faith/Science from: 1) academia, 2) industry.
I-B	Perspectives on Faith/Science from: 3) campus ministry, 4) church organization.
II	Literature, sci/tech, nature and beauty (is there such literature, or is beauty always perceived as "natural"?).
III	Beauty in: 1) medicine, 2) law, 3) language, architecture.
IV	Smaller groups: how do I find beauty in my work and life and how do I integrate it into my faith life?
V	Spirituality (faith in action) and the beauty of work.
VI	Environment/science/faith/beauty (a reprise).
VII	Suggestions for ITEST's next 25 years.

A 45-minute ecumenical worship service, using various forms of beauty (art, literature and music) will be held at 5:45 p.m. on Wednesday, August 4, 1993. This service is one of thanksgiving for God's beauty and his gifts to us of beauty in each other and in the world around us.

ABOUT THE LECTURERS

Fr. John Staudenmaier, S.J. is Professor of the History of Technology at the University of Detroit - Mercy in Detroit. A prolific author, Fr. Staudenmaier is a well-recognized expert in a field of growing importance.

Mr. Leonard Buckley is Foreman of Designers at the Bureau of Engraving and Printing in Washington, D.C. Among his works are two covers for ITEST Publications (Perspectives on Creation and the Vineyard). You have seen his skill in the United States' Apollo 8 stamp as well as the currently used mineral stamps.

Most Reverend John Sheets, S.J. is Auxiliary Bishop of Fort-Wayne - South Bend. Bishop Sheets has served as Professor and Chairman of the Theology Department at both Creighton and Marquette Universities.

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Mennonite Central Committee

IN MEMORIAM

The Most Reverend Cletus F. O'Donnell
Bishop of Madison, Wisconsin

We ask your prayers for Bishop O'Donnell. We also request that you remember several ITEST members who are seriously ill.

HOMILY FOR ACADEMIC MASS OF THE HOLY SPIRIT

Fr. Martin Palmer, S.J.

College of Philosophy and Letters
St. Louis University, 28 Aug. 1992

As we begin our year of studies to be ministers of the Gospel, it's important that we try to have some sense of the situation in which we find ourselves and of the future we are preparing for. This holds for teachers no less than students.

All times are remarkable — the question is, what is remarkable about ours? I think our time is dominated by the unexpected emergence of two enormous crises, both of which have darkened for us the horizon of the future.

The first crisis is the crumbling of ideological humanism that we have seen in the recent self-destruction of communism. The second is the realization that our exploding science and technology, far from guaranteeing us a happy future, may fail even to stave off the worsening of the human condition, a worsening to which technology itself has substantially contributed, both directly and indirectly.

Beginning about 1600, European culture, which was eventually to dominate the globe, began to develop apart from Christianity and even against Christianity. Partly this was a skeptical reaction to the wars of religion after the Reformation, but there was more. The real driving force was a burgeoning ideology: an exalted idea of human reason and power, a belief that, once come of age and freed from superstition, humanity would be able to produce a material, cultural, political world that surpassed our deepest longings.

We now find ourselves hoping at most that science and technology may be able to mitigate the damage as the planet and humanity careen out of control. The most evident symbols of this change are the destruction of our ecology and the AIDS pandemic.

In the nineteenth century these ideas became powerful to a degree that is almost inconceivable to us today, and they ushered in the vast and tragic ideological movements of the twentieth century. These ideas and these movements have been a constant and often overpowering *rival* to Christianity as a source meaning and purpose. Christianity has been everywhere marginalized; and, when it was not, it often betrayed itself by desperately trying to come to terms with these ideologies and movements.

Now we have witnessed the dramatic implosion and collapse of European communism and the revelation that the only real power it ever had was to create human misery on an almost unimaginable scale. But the fall of communism has an even greater significance. Communism was the last and the most powerful of the humanistic ideologies. Its demise brings with it a clear sense that humanity is now all out of "great ideas." Shattered bits of humanistic theory still dominate our culture — but that is precisely why our culture seems adrift, with nowhere to go.

At the same time, the 19th and now 20th centuries produced exponential scientific and technological growth. Until just a short while ago, we all assumed that this progress would in some way vindicate human nature and bring human happiness and fulfillment. That has now dramatically changed. We now find ourselves hoping at most that science and technology may be able to mitigate the damage as the planet and humanity careen out of control. The most evident symbols of this change are the destruction of our ecology and the AIDS pandemic. We are both socially and scientifically helpless in the face of these, and will likely remain so as they progressively devastate whole regions of the globe, whole sectors of humanity. People who wrote about science or technology even when I was in college would never have envisaged our present situation.

The upshot of all this is that Christianity meets a very different world today from what it met a hundred or even fifty years ago. Christianity has less need to fight, as it did in the past, against rival hopes and ideals, rival programs for human fulfillment. It also has less excuse for compromising with these hopes and ideals, as it has so often fatally done in the past. The life has gone out of the ideologies, and the human race is experiencing something like a vast hangover, as it is confronted face-to-face with its terrible limitations and the devastation it has wrought on itself.

For the future, humanity's great temptation will not be

to attractive ideas and ideals. In the face of human limitations and suffering, the great temptation will be for the few who have to do whatever it takes to keep what they have, while the many who have not will strive to get it. The great temptation will be exactly what St. Paul spoke of when he said that, if the gospel of Christ's resurrection and ours is not true, then "eat drink, and be merry, for tomorrow we die." The future of the human race, on its own, no longer has anything magical or attractive about it at all. There will be no glorious ideas and ideals to mask the ugliness of what is going on.

Our task is in the coming century will be less to find ways of fitting Christianity and the Gospel together with any of the humanistic ideals lying in ruins around us, than to learn how to live the gospel at white heat right out of its source in Christ, and to preach the gospel in all its integrity as light for a world which knows it is in darkness.

And so our preparation as proclaimers of Christ demands, as I see it, three things.

First, of course, each of us needs to acquire the skills needed for operating effectively in our society: thinking skills, communication skills (speaking, writing), and general "cultural literacy. But much more deeply, we also need to study history, science and philosophy so that we can understand how the world we live in was made, how it reached its present pass, and what resources, if any, it has for facing the future.

But above all we need to study Christianity in as deep and centered way a possible. We need to study Scripture and whole Christian tradition with the constant and overriding question before us: *What is the main point? What is the heart of it all? What is Christianity at its fullest, purest, most uncompromising?* Our task is in the coming century will be less to find ways of fitting Christianity and the Gospel together with any of the humanistic ideals lying in ruins around us, than to learn how to live the gospel at white heat right out of its source in Christ, and to preach the gospel in all its integrity as light for a world which knows it is in darkness. And out of our Christian faith, we must be ready to bring joy and persistence to our participation in the struggles that lie ahead for the human race.

In a way, after the fifteen-century rise and fall of Christendom, and the five-century rise and fall of the humanistic era, we are back at the starting-gate. The field is once more level. In the twenty-first century just as in the first century, we in the Church will be challenged to present the gospel of Christ anew in all its intensity, in all its purity, to a world whose only alternative will be a mean and despairing existence. To be agents of this will be our privilege and responsibility. It will take hard work to know the world we are living in, and even harder work to recover—with our minds and with our hearts and together as a Church—the intense reality of the gospel that once conquered the world. But in this work Christ himself will be sustaining and strengthening us, by His Spirit, which in our day too is reaching out to the ends of the earth.

THE HUMAN GENOME PROJECT:
Molecular Methods and Applications

Peter J. Leonard, OSFS

This paper was prepared for the Oct., 1992 ITEST Workshop on the Human Genome Project.

The material which I am about to present is a brief summary of the methods involved in sequencing DNA and the application of these methods to the human genome project. This is a difficult task when I consider both my time and length constraints, as well as the wide assortment of expertise present at this 1992 ITEST conference. To this end, I will endeavor to present this topic with an eye toward an audience which has little or no experience in the biological sciences.

In reality, most of the biotechnology employed in the human genome project has been well developed for the past ten years. The major innovations have occurred in two areas of automation: mechanized methods of sequencing and computerization of sequencing data. Automated sequencing relies basically on the techniques that I will outline below and only represents a savings in the amount of human energy expended. Computerization, which will be addressed during the conference by Mr. Gary Menard, SJ, represents a

means to enable biologists to better handle the enormity of data generated by this area of research. Considering both the nature of our audience and the fact that little has changed in the actual methodology of gene sequencing, I thought it would be most effective to present highlights of material that is primarily reviewed in the text: Molecular Biology of the Gene, by Watson *et al.* This text is less technically written than the original research articles which outline the basic technology, while providing a good reference base for those whose expertise invites them to explore further. In areas of recent development, I have referenced more specific articles.

A. DNA, the Basis of Genetic Transmission

Since the early 1950's, scientists have known the structure and identity of *D*eoxyribo*n*ucleic *a*cid (DNA), as the sole molecule responsible for the transmission of genetic information in bacteria through higher organisms including humans, (See Watson, *et al.,* 1987, pp. 68-82). The ability of DNA to transmit genetic information rests in the linear arrangement of four nucleotide bases, namely Adenine (A), Thymine (T), Guanine (G) and Cytosine (C). DNA is a duplex molecule with two strands exhibiting complementary base pairing. "A" pairing with "T" and "C" pairing with "G." Replication of DNA is accomplished by separation of the two strands and synthesis of a new complementary strand, a phenomenon known as semiconservative replication. Polypeptides, the constitutive element of proteins, are also linear molecules composed of amino acids. The linear arrangements of amino acids in a polypeptide molecule is indirectly specified by way of a triplet code based upon the DNA bases mentioned above. Since DNA is located exclusively within the nucleus of higher cells and polypeptides are synthesized within the cytoplasm (the non-nuclear region of cells), communication must be affected by an intermediate molecule. This molecule is known as *R*ibo*n*ucleic *a*cid (RNA). The particular class of RNA which communicates the DNA code between nucleus and cytoplasm is known as messenger RNA or mRNA. DNA serves as a template for mRNA, and mRNA communicates this code to the cytoplasm, where the amino acid sequence of a polypeptide is interpreted: one amino acid for every three nucleotide bases. This relationship between DNA sequence, message sequence and polypeptide composition is known as the "Central Dogma of Molecular Biology," and explains the relationship: DNA sequence specifies the mRNA sequence, which ultimately specifies the order of amino acids found in a polypeptide. A gene, may then be defined as a region within a DNA molecule responsible for encoding a polypeptide.

For the non-biologist, it is often difficult to understand the relationship which exists between the sequence of DNA and the assembly of a higher ordered structure such as a cell. While it would be impossible to treat this subject completely here, it is revealing to note that proteins are necessary for a myriad of structural and functional roles. On the structural level, everything, including hair, nails, tendons, etc. is composed primarily of proteins. On the functional level, enzymes are composed of protein. All metabolic functions are controlled by enzymes, including those responsible for the production of cellular components. Non-protein molecules such as lipids (fats), can be synthesized and degraded by way of enzymatically governed processes. During the embryonic period, most of the signals for the development of an individual are protein based. Some of these signals are even laid down maternally, prior to fertilization. Thus all of the functioning of life as we know it is dependent ultimately upon DNA sequence.

Since DNA sequence is so important to life, it follows logically that alterations in DNA sequence can have equally disastrous effects. Mutation, or an alteration in the base sequence of DNA, can result from a change in as little as one DNA nucleotide base. This alteration leads to a change in the amino acid sequence of a protein. Diseases such as Sickle Cell Anemia and Cystic Fibrosis can be traced to a single change in the amino acid sequence, (See Collins, 1992). More extensive rearrangements of DNA can also result in mutationally affected diseases. Thus, the utility of relating DNA sequence to the determination of human genetic diseases should be clear, at least at a technological level.

B. Problems Encountered in the Handling of DNA

DNA is an enormously long molecule. In humans, the size of the human genome has been estimated to be 8 x 10^9 base pairs (bp), (See Watson, 1987, p. 272). It is impossible to isolate intact DNA from higher organisms since it will tend to shear or break. When a biologist attempts to isolate large amounts of DNA from different cells, the DNA will tend to shear at different locations, creating an assortment of broken DNA molecules of varying length, a condition which is almost impossible to resolve. The problem of length is further compounded in higher organisms by a phenomenon where most genes are not encoded in a continuous format. Instead, the coding regions of genes, known as introns, are interrupted by non-coding regions known as exons. Thus, both introns and exons are transcribed, but the RNA is processed to remove exons

before it leaves the nucleus as message. The most obvious problem which this presents is that the genes from higher organisms are much larger at the DNA level than at the level of message or resultant amino acid sequence. Two developments, restriction digestion and production of cDNA, have advanced our ability to deal with these problems. Both of these methods will be explained in the section which follows.

C. Methods of DNA Preparation and Isolation

The technique for sequencing DNA was revolutionized from the mid 1970's through mid 1980's when enzymes known as restriction endonucleases were first discovered, (See Watson, 1987, pp. 266-269). These enzymes, isolated from various strains of bacteria, are capable of recognizing specific series of base sequences in double stranded DNA and cleaving the molecule at a site adjacent to the sequence recognized. Numerous restriction enzymes have been purified, each capable of recognizing sequences of DNA ranging from 4 to 10 base pairs (bp). Since DNA is composed of only four different nucleotides (A,T,C,G) and since restriction enzymes are capable of recognizing short sequences of DNA, it is statistically likely that an enzyme recognizing a 4 bp sequence of nucleotides will do so at a rather high frequency and an enzyme which recognizes a 10 bp sequences will do so less frequently. In fact, the predicted frequency is given by the relationship 4^n, where "n" represents the number of bases recognized by the enzyme and "4" having been derived from the number of possible nucleotide bases (A,T,C,G). Thus, an enzyme that recognizes a 4 bp sequence would tend to cleave at 256 bp intervals, an enzyme that recognizes a 5 bp sequence would tend to cleave at 1024 bp intervals, and so on. The problems mentioned above, that result from the length of the DNA molecule, are circumvented by cutting the molecule into smaller fragments at regular intervals. Thus, larger amounts of DNA can be isolated with cuts at identical intervals.

In lower organisms, such as bacteria, the fragments of DNA which result from digestion can be separated directly by electrophoresis on an agarose gel, (See Watson, 1987, pp. 270-271). This process separates the fragments on the basis of their size: with longer fragments moving more slowly through the gel matrix than larger ones. This technique is sufficiently accurate to estimate the length of the DNA with an error rate of less than 5%. Once separated, individual sequences of DNA can be removed for sequencing.

In higher organisms, additional complexity is encountered. The genome size of humans can potentially produce over a million different fragments of varied sizes, far too many for resolution on an agarose gel. The solution to this problem rests in amplification and isolation of the gene prior to sequencing, (See Watson, 1987, p.596). This is accomplished by insertion of restriction cut fragments into an independently replicating structure, usually within a bacterium or phage. When the complete DNA of an organism is fragmented and inserted into an independently replicating structure, a gene library is produced. Until recently, three major vehicles were used to amplify DNA: Plasmids which are effective at integrating small segments of DNA ranging from 5-10 Kilobases (Kb), Phage lambda systems which require 22 Kb fragments for effective packaging, and cosmid vectors which hold 40-50 Kb of DNA. All three of these systems presented major difficulty, since human genes are often 100's of Kb in length. In 1987, Yeast artificial Chromosomes were devised to solve this problem.

Yeast artificial chromosomes (YACs) provide a vehicle for cloning large segments of exogenous DNA (Burke *et al.,* 1987 and especially Shero, *et al.* 1991). While fewer in number, endogenous yeast chromosomes are approximately the same size as human chromosomes and thus serve as efficient vectors for the amplification of human genes. The chromosomes of higher organisms possess the following common features: centromere, replication origin and telomere. YACs are constructed by splicing foreign DNA into a yeast derived vector containing the three necessary elements mentioned above. The end product is a YAC which will faithfully clone inserts of up to 10^6 bp. YACs are particularly useful when one is attempting to clone a large gene with many exons, and offer considerable promise in allowing even larger regions of DNA amplification, an innovation that will make human genome sequencing far more efficient.

An additional recent development for gene amplification is the Polymerase Chain Reaction (PCR), (For an interesting review, see Mullis, 1990). This technique allows the direct amplification of an isolated DNA region. Similar technologically to the Sanger method (see below), PCR mimics the natural process of semiconservative replication *in vitro.* The uses of this technique are only beginning to be discovered.

The problems created by the existence of exons can be circumvented using the enzyme reverse transcriptase, (See Watson, 1987, pp. 609-614). In many cases, it is possible to isolate the mRNA for a particular gene. Since mRNA has been processed to remove exons, it is

truly representative of the coding region of DNA. A virally derived enzyme known as reverse transcriptase can be used in the laboratory to produce DNA from the isolated RNA message. When DNA is produced from a particular mRNA, it is known as cDNA (meaning complementary DNA), and is of considerable utility as we will see later in this paper. Prior to the development of YACs, the principle method of amplifying human genes was by generation of a cDNA followed by incorporation into a phage lambda system. Looking ahead to the future, the phage lambda system will most likely remain the principal method of amplifying cDNA derived clones, while YACs will offer the principal means of amplifying human genes when it is desirable to use nuclear DNA with exons intact.

In general, there are two approaches to the human genome project. In the first approach, the goal is to obtain the sequence of the entire human genome. This involves the sequencing of the entire genomic library produced from human DNA. The advantage of this approach is that it will produce complete sequence data, including all exons and other intervening repeat regions. This is an immense undertaking which has the liability that it does not immediately reveal the location of each gene. The second method, which I will detail below, involves the sequencing of each gene encoding region from cDNA. This method has the opposite liability in that it will not reveal the sequence of exons and repeating regions. The maximum utility of the human genome project will come from a marriage of data obtained from both directions.

In addition to genomic libraries, it is also possible to produce a cDNA library, (See Watson, 1987, pp. 611-615). Such libraries, while more difficult to produce than a genomic library, possess the added advantage of being confined to sequences which are known to be expressed. The cDNA regions are often less difficult to screen since they possess the ability to be expressed in bacterial systems. (Recall that exons exist only in higher organisms. While bacteria do not have the ability to remove exons, this will not be a problem if cDNA is used.)

This leads us to an interesting problem, one that remains the most difficult task for molecular biologists: How does one correlate a gene with its function? On the genetic level, the most basic approach is a technique called complementation, (See Watson, 1987, p. 218). Complementation begins with cells that are deficient in the ability to produce the gene product of interest. These cells may be obtained naturally or may be generated rather easily by mutating a population of cells. Once obtained, isolated DNA may be absorbed or transformed into the deficient cells. If the normal gene of interest is successful in rescuing or complementing the deficient function, a correlation may be made between a segment of DNA and its function.

Digested DNA may also be incorporated into an expression vector, (See Watson, 1987, pp. 614-615). In this situation, an entire cDNA library can be incorporated into bacterial DNA. If incorporated into the appropriate region, the bacteria will begin to produce the gene product. The bacteria can be plated as single cells and then screened using various techniques such as interaction with a specific antibody. The bacterial clones can then be separated based on their ability to produce the gene product. The DNA, whose gene product has been identified, is now available for sequencing.

D. Methods of DNA Sequencing

Once DNA has been digested into smaller fragments and incorporated into an amplification construct, it can be amplified, isolated and sequenced. There are several methods generally employed; the most common is known as the Sanger method (see Watson, *et al.*, pp. 274-276). This method relies on the enzymatic synthesis of a complementary DNA from an isolated single stranded DNA. The DNA is synthesized with a small amount of a dideoxy analogue added to a reaction mixture containing all four normal nucleotide bases (A,T,C,G). This analogue causes a termination of synthesis along the newly synthesized strand at any point where it is incorporated. Initiation of synthesis can only take place at the origin of the fragment due to the necessity of supplying a primer. Since the analogue is supplied in a relatively small proportion, strand termination will occur at any random position where the analogue is incorporated. Theoretically, various DNA strands will be produced of lengths corresponding to the position of the particular nucleotide. Upon separation of the single stranded DNA on a polyacrylamide gel (capable of distinguishing even single base pair differences), the position of the particular base can be inferred by the length of the strands. For example, if a small proportion of dideoxy-cytosine (ddC) is added and C is located at positions 1,3,9,11 and 13, then single stranded DNAs will be produced with lengths of 1,3,9,11 and 13. If DNA synthesis is then conducted on the same template with dideoxy-adenine added, newly synthesized strands will be produced with lengths of perhaps 2,4, and 12 bp. Repetition of the procedure with all four dideoxynucleotide bases will decode the entire length of newly synthesized DNA.

E. Relating DNA Sequences to Mapping and Function

As elegant as the above techniques appear, the clinical utility of DNA sequencing will remain nonexistent unless the actual sequence of DNA can be related to its location and function within the genome. At this point, it would seem particularly helpful to make the distinction between sequencing and mapping. Sequencing, as outlined above, consists of a determination of the order of bases in a particular region of DNA. The practical utility of sequencing is twofold: it is useful to know the exact sequence of DNA bases which results in the production of functional protein. Second, determination of defective gene sequence permits diagnosis by comparison and offers the beginnings of a gene therapy. In addition to the value of particular gene sequence, the spatial relationship or location of genes is also informative. This leads us to an additional genetic enterprise known as mapping.

Mapping involves the assignment of location of a particular gene within the genome of an individual. Useful data is often absolute or relative. Beginning with Gregor Mendel during the previous century, it has long been known that certain genetic traits tend to be inherited together, a phenomenon known as linkage. On a cytogenetic level, linkage is the result of two genes coexisting on the same chromosome. While individual chromosomes may become assorted between generations, the genetic information located on a single chromosome is inherited as a unit. Prior to the advent of molecular biology, determination of linkage was a primary means of mapping particular genes to particular chromosomes.

The human genome consists of 22 pairs of homologous autosomal chromosomes and one pair of sex chromosomes. Another long discovered genetic phenomenon known as crossing over occurs between chromosomes of a homologous pair. While exchange is relatively rare, it has been determined that the probability of exchange is directly dependent on the distance of gene separation. While linkage is used to determine the coexistence of two genes on the same chromosome, crossing over data can be used to determine the relative proximity or "map distance" between linked traits. Some genetic defects result in anomalies which can be visibly identified by way of isolating human chromosomes and observing them on a light microscope (karyotyping). Prior to the advent of molecular biology, visible identification of genetic defect was the principle method of assigning genes to a particular chromosome. Once assigned, other genes could be assigned to the same chromosome as a result of linkage. Finally, the distance between genes could be assigned from the crossing over data. Compared to modern techniques, these particular methods of mapping were far more laborious and required a certain degree of luck in finding identifiable traits.

Molecular advances have revolutionized our ability to map the location of human genes. Interestingly, however, molecular techniques have also confirmed much of the data which was determined by the earlier methods. Structural DNA, which is non-coding, can be identified by hybridizing single stranded radioactively labeled RNA *in situ* to the particular region, (See Watson, 1987, p. 667). The exact position of this DNA known as Satellite DNA, can be identified on metaphase chromosomes via a technique called autoradiography.

Specific gene locations are more difficult to locate. Most often a technique known as Chromosome Walking is employed, (See Watson, 1987, p. 616). This technique, which is most useful in analyzing longer stretches of DNA, utilizes overlapping stretches of library derived DNA to produce a composite sequence. Also, sequencing data obtained from known genes can be reconciled with extensive computer databases which include known sequences of DNA that have not been assigned an exact gene identity, (See Watson, 1987, p. 277).

Related gene sequences may often be isolated via hybridization techniques, (See Watson, 1987, pp. 608-609). Often genes with related functions possess related sequences. A good example of this is myoglobin, found in muscle cells, and hemoglobin, found in red blood cells. Both gene products are responsible for carrying oxygen. Single stranded DNA from one sequence will tend to bind to the other. The resultant hybrid can be tagged within a library for isolation. Relatedness can also be determined using a technique called Southern Blotting. This technique relies on separation of DNA on an agarose gel. Once separated, the DNA is transferred to nitrocellulose, where it can be probed with complementary DNA or RNA. Only identical or related sequences will bind and are thus easily identified.

The mere size of the human genome makes the determination of total sequence a mammoth undertaking, both in terms of hours and expense. Besides the philosophical value of knowing the entire sequence, two additional clinical advantages are potential rewards. The first is in the area of diagnosis. Since the determination of gene sequence usually involves methods of hybridization, which is highly sequence

specific, numerous diagnostic assays will be possible as a result of this research. The second area is gene therapy, (For a review, see Anderson, 1992). The knowledge of correct and mutated sequences opens the door for the correction of genetic error. Various obstacles stand in the way before this can be a reality. An effective therapy will need to have a reliable means for delivery of the correct DNA to the appropriate tissue. Integration of the exogenous DNA into the host cell will also present an obstacle. Some of these obstacles are already being overcome, at least within the laboratory setting. As intriguing as these benefits seem, my task in this paper is complete. At minimum, the power of the genetic techniques already developed should convince us that we all have something rewarding to which we can look forward.

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