Molecular Biology and Gene Cloning: Thinking the Thoughts of God

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ABSTRACT

A review of the techniques used in molecular biology reveals that all of them primarily utilise naturally existing life systems. Genetic cloning and manipulation are similar to organ transplants in medicine and grafting in botany. Molecular biologists use existing genes, proteins and enzymes produced by life to make new combinations and modifications. This field, rather than supporting evolutionary naturalism, eloquently supports the creation worldview and provides massive evidence for intelligence and design.

INTRODUCTION

This review discusses some commonly used techniques in molecular biology to illustrate the similarity between these methods and other areas in which humans have used materials that already exist in nature and have employed them for our own ends. An analogy is a child playing with Lego™ blocks or an erector set, manipulating the parts according to how the set was designed to be used. As an erector set can be used in ways for which it was not intended, humans can likewise manipulate the genome in ways that are harmful, a fact well recognised by modern geneticists. Consequently, much time has been invested to evaluate the ethical ramifications of the many genetic procedures currently being used.[1]

Discussions about the revolution in molecular biology in the mass media often imply that researchers have now unlocked the secret of life, and can create life or significantly modify it. Book titles including **Playing God** by Goodfield, **Come, Let us Play God** by Augenstein, and **Brave New People** by Jones reflect this concern. The fear both inside and outside of the biomedical community that human tinkering with genomes may create undesirable life forms — such as supergerms that are immune to all existing natural and laboratory defences — has been pervasive for years.[2]

These fears have proved to be largely — but not totally — unfounded, and molecular biology is now a major scientific enterprise. Its techniques are commonly used in every life science area, from bacteriology to sports medicine to pathology and even criminology. This brief review focuses on how gene manipulation is done, which helps us understand why these fears have existed and why some still exist.

The molecular biology techniques used are largely those which **already exist** in nature, and use of them is more comparable to our exploitation of livestock to breed meatier cows than creating new life forms in the Frankenstein tradition. Nonetheless, even animal breeding poses dangers, as the example of the human breeding of the killer bee illustrates. Likewise, it was feared that organisms such as bacteria could be produced by DNA technology which could not be controlled by existing antibiotics or the human immune system.

Some persons fear that human gene manipulation by recombinant DNA technology and artificial manufacture of a gene segment that can be inserted in the genome supports a mechanistic, non-theistic interpretation of life. They reason that if humans can assemble a gene, then creating life cannot be the domain of God. However, because a copyist can accurately reproduce the arrangement of letters in a Shakespearean play does not make the copyist's ability equal to the original author's. Copying is not creating, but is a mechanical process which requires few skills except accuracy. Further, the techniques that researchers use to manipulate genes are actually for the most part tools that are part of the original creation. Living

systems cannot be manipulated to any significant degree without using gene components from other living systems.

What humans have achieved by biotechnology is not unlike a householder adjusting his VCR by using the control knobs designed by the manufacturer. Almost all of the enzymes, structures, compounds and systems humans have discovered, and learned how to apply in recombinant DNA research, already exist in nature.[3]

ENZYMES AND LIFE

Among the most important structural units necessary for life are enzymes. They are extremely complex, typically quite large macromolecules which catalyse certain chemical reactions. All living organisms use them extensively in order to control the chemistry of life. Many poisons, as well as heat above a certain temperature, are fatal because they distort enzymes, prohibiting them from working. The number of enzymes in a human being is estimated to be as many as 100,000. A catalogue of some of the more important and common types of enzymes, which illustrates the wide variety of functions, is found in Table 1.

It is still far beyond the ability of scientists to design from scratch an enzyme that would carry out processes such as DNA polymerisation. If such an enzyme could be designed, it would be possible only after an intensive study into how the DNA polymerase that God created works, and the finished product would likely be a very close copy of what God had already created. Scientists have been able to alter the activity of some of these DNA enzymes so that they better suit the needs that they have in the laboratory, but this is a long way from designing them from scratch.

1.	**OXIDOREDUCTASES**	Catalyse oxidation-reduction reactions.
2.	**TRANSFERASES**	Enzymes which catalyse a transfer of a group of atoms either from one substrate to another or from one part of a substrate to another part.
3.	**HYDROLASES**	Cause hydrolysing reactions. They catalyse the hydrolysis of anhyrides, amides, imines, esters, etc.
4.	**LYASES**	Catalyse the non-hydrolytic removal of groups to form a double bond or the addition of groups to a double bond.
5.	**ISOMERASES**	Catalyse isomerisations, or the conversion of one isomer to another, and **mutases,** enzymes which cause the rearrangement of atoms within molecules.
6.	**LIGASES**	Catalyse hydrolysis reactions such as between adenosine triphosphates (ATP) and other nucleoside triphosphates with covalent bond formation.

***Table** 1. The common enzyme families.*

RESTRICTION ENDONUCLEASES

All of the techniques described in this paper involve primarily an exploitation of features in the existing natural DNA system. A major example is the important tool used in cutting DNA and RNA strands for research work. This tool is a complex protein called a restriction endonuclease (RE), or restriction enzyme for short. Restriction enzymes are endonuclease enzymes extracted from bacteria. Endonuclease cleaves nucleic acids at **internal** positions (away from the ends of the DNA strand), and exonucleases progressively disassemble DNA from the **ends** of nucleic acid molecules. These complex protein structures are obtained by purification from bacteria or by recombinant DNA methods.[4]

Bacteria lack an immune system and have only one major defence against the bacterial virus family called bacteriophages. These viruses attack the bacteria by inserting their DNA into the bacterium, which then takes over the bacterium's cell structures to construct more viruses. The bacteria defend themselves by cutting the invader DNA into small pieces using restriction enzymes. Most common restriction enzymes function by catalysing a hydrolysis reaction that uses water to break a specific phosphodiester linkage on each strand of the DNA helix.[5]

Three basic classes of restriction endonucleases exist. Class II cuts **only** a very **specific 4** or 6 base pair sequence, and many will cut **palindrome** sets, such as GTA and ATG, in order to degrade the invader DNA. Type I cuts at **random** sites about 1,000 nucleotides away from its recognition sequence, and Type III cuts at sites **near** the recognition sequence, but **exactly where** is difficult to predict. Restriction endonucleases do not 'progressively' degrade DNA — once they make a cut, they will **not** continue to cut. Type I and III restriction enzymes require ATP for energy to cut DNA, and type II needs only magnesium ions (Mg^{++}) as a co-factor. Over 1,200 kinds of restriction enzymes are now believed to exist, and these recognise about as many different nucleotide sequences. About 100 kinds are now commercially available, and more are introduced each month. This variety is critical for DNA work because it allows cuts to be made in very specific areas of DNA. It also allows DNA mapping, is critical in DNA fingerprinting and is necessary for disease identification.

The names of restriction endonucleases used in research are derived from the genus and species of their source. The first restriction enzyme isolated was achieved in the 1970s by Smith and Wilcox.[6] It was from *Escherichia coli* and is named *Eco* Rl (R is the bacteria strain, and '1' means this was the first restriction enzyme discovered from this strain). It cuts between the G and A in the sequence GAATTC as shown by the asterisks in this position G*AATTC. Other specific types of cuts include:

(1) Bam HI which produces a staggered cut so that a 5-prime section hangs over each end. It cuts at G and G in 5'G*GATCC 3' and 3' CCTAG*G 5' (representing

the complementary DNA strands).

(2) Pst 1 leaves 3' overhanging on each end and cuts at 5'CTGCA*G 3' and 3' G*ACGTC 5'.

(3) Other enzymes, such as *Hind II,* leave a **blunt end** cut, that is, with no overhang, as in CC*GG and GG*CC.

Non-blunt end cuts can be used to achieve directional cloning, where a gene can go in the plasmid only one way which is necessary for it to function. A restriction map lists all of the locations where restriction endonuclease cuts can be made on a plasmid or DNA strand. Most recognise only 4, 6 or 8 bases, but one super-rare cutter has a recognition site of 18 bases! Without these large complex proteins which can cut at precise locations in DNA molecules, most of the manipulations now done in labs would be impossible. A study of these enzymes gives us a greater appreciation for just how wonderful and complex God's creation is.

An organism obviously must not restrict (cut) its own DNA because this would be lethal. Consequently, to prevent this it tags its own DNA, a protection method called a host restriction modification system. The protection involves methylation, the binding of a methyl (CH_3) group to the base that is cut by the restriction enzyme. Methylation of one strand is referred to as a hemi-methylated state. Methylation usually **blocks** cutting due to the methyl group being bonded to the adenine base in the DNA strand, which prevents the restriction enzyme from physically positioning itself in a place to cut. Removing these protectors from the restriction endonuclease cleavage sites allows restriction enzymes to make a cut there. Some viruses produce enzymes that degrade methylations, thus they destroy this normally effective self-protection of the bacteria.

THE PROCESS OF GENE CLONING

Moving one gene from one organism to another, such as an insulin gene into a bacterium, is a problem in that many genes are extremely long, creating cloning difficulties. We know that as many as 95 per cent of the base pairs in a gene are not necessary to make the protein of concern. Fortunately, all these base pairs are removed by complex genetic mechanisms. After a copy of DNA is made (called messenger or mRNA), it is edited extensively, producing a processed form which has only the base pairs needed to make the protein. Instead of cloning the DNA itself, it has been found to be far more efficient to use the processed mRNA to make a DNA copy, called copy or cDNA.

The cDNA is made by reverse transcriptase, an enzyme which copies RNA and makes the copy into DNA. Reverse transcriptase was originally discovered in viruses called retroviruses. Most organisms store their genetic material in the form of DNA, but a few viruses use RNA as the master blueprint which must be converted to DNA, then back to RNA, to produce the messenger RNA necessary to manufacture the needed proteins.

The cDNA cloning technique first requires that we either grow the cells which contain the genes of interest, or obtain a sample of tissue or of blood as a source. The next step is to isolate and purify the mRNA which exists in the cell. The common means of purification is by affinity chromatography using oligo(dT). Oligo(dT) is a tail of single stranded poly-TDNA which has been developed to help purify the mRNA. Next, the mRNA that is desired is located, and reverse transcriptase is used to make a copy, producing cDNA. A complementary strand is then made called second strand cDNA, producing a duplex. The duplex then can be amplified (copied many times) by polymerase chain reaction (PCR).

PCR is a method where a single duplex DNA can be duplicated to produce millions or even billions of copies. The reaction uses two oligonucleotide primers that hybridise (bind) to opposite DNA strands, flanking the target DNA sequence to be amplified. A primer is a single short strand DNA which binds at each end of the separated strands to allow the process of copying to begin. Primer elongation is catalysed by the enzyme Taq DNA polymerase, which is called heat stable because it is stable at temperatures necessary to 'melt' the DNA (meaning to separate the duplex). The source of this unusual enzyme is a thermophilic eubacterium called *Thermus aquaticus.* Essentially the strands are separated by heat, then allowed to cool so that Taq polymerase can make a copy (it works only when it is cool). The strands are then heated again, causing melting of the DNA. They are then cooled again, allowing another set of copies to be produced, approximately doubling the amount of DNA each cycle.

To melt the DNA, the temperature of the PCR unit has to be raised and then lowered so the copy can be made. Each time a new DNA polymerase is added because the heat would destroy it, a very time consuming process. Then the heat stable DNA polymerase noted above was discovered in the thermophilic bacteria found in the hot springs at Yellowstone National Park. These bacteria are active in temperatures over 70°C and stable at 95°C, but are not destroyed at this temperature. Consequently, raising and lowering the temperatures can denature the DNA, breaking the hydrogen bonds between the base pairs (a process called melting) without ruining the enzyme and requiring its replacement as previously. The heat cycles are repeated, causing denaturation, primer annealing and extension of the end primers by the Taq DNA polymerase.

The products of each cycle serve as a template for the next cycle, producing the target DNA copying, approximately doubling each cycle (1 to 2, 4, 8, 16, 32, etc.). PCR can be used to amplify as little as a single DNA molecule to over a billion copies in about three hours. It has been made famous by its use in criminology, but is a standard technique today in molecular biology.

Next, the copied cDNA duplex strands are ligated (spliced) into a vector (carrier) by use of an enzyme called T4-DNA ligase. The vector can be any DNA entity which the target cell will accept. A commonly used vector is

lambda bacteriophage, but plasmid and/or cosmid vectors are also common. Next the vector is introduced into the *E. coli* bacterium or another living system. This results in a small number of successful clones which must be selected. Typically used to detect the successful expression of the DNA of concern is a radioactive or luminescent labelled DNA or RNA 'probe'. Another method is to use an antibody to detect the presence of the protein product. RNA and DNA labelling requires the use of enzymes to cause the strand to copy, such as one called a Klenow fragment, which is part of the DNA polymerase I enzyme.

OTHER ENZYMES COMMONLY USED

Many thousands of enzyme types exist, all of which are naturally produced by living organisms.[7] Enzymes used for degradation of cellular components (necessary to repair and replace cell parts) include proteinase K which digests native proteins. It is used to inactivate endogenous nucleases such as RNases and DNases, to protect the oligonucleotides (DNA or RNA) as they are being purified. DNase is used to degrade DNA, leaving only RNA in the sample. Likewise, RNase is used to break down RNA and leaves only DNA. Agarase is used to break down the agarose gel in order to recover most of the DNA that is sorted in an agarose gel. Once separated by strand size, it can be physically cut out of the gel, then the agarose broken down by agarase to purify the DNA contained in that section.

Lysozyme is another enzyme useful in plasmid preparations to break down the cell wall and the outer membrane. Other enzymes used in cloning include polynucleotide kinase which catalyses the transfer of the terminal phosphate group of ATP to the 5-hydroxyl ends of DNA or RNA. This is useful for adding radioactive labels to DNA and RNA molecules. RNA polymerase catalyses the RNA synthesis that is DNA-dependent. Terminal transferase catalyses a template-independent addition of dNTPs (deoxynucleotide phosphates) to the 3'-hydroxyl group of DNA without a template present. This is useful for adding homopolymer tails to DNA fragments in cloning experiments. This segment, containing several of the same kind of nucleotides, is a method used to join double stranded DNA by nucleotide transferase.

USE OF PLASMIDS AS VECTORS TO TRANSFER GENES

Plasmids are circular, double-stranded DNAs that autonomously replicate in the cell separately from the chromosome. They are used by bacteria for purposes that include protecting themselves from environmental toxins and pathogens. Plasmids also direct the manufacture of toxins to defend themselves from enemies, carry the genes that cause the degrading of toxins, provide resistance to antibiotics, and allow the cell to use a particular substrate.

Each bacterial cell may have one or two large plasmids and 20 to 500 or more copies of smaller ones. Their lengths range from 1.5 kilobases (kb) to over 300 kb.[8] The smaller plasmids have about 5,000 nucleotides, which is about the number needed to produce five average-sized proteins. Usually about 1,000 nucleotides code for one bacterial protein.[9] *E. coli* has a total of about 5 million base pairs, and human DNA has an estimated 3 billion base pairs. Plasmid DNA types include monomer, catenated dimer (when two plasmids are interlooped), single-stranded DNA (found in some viral DNA types) and supercoiled, when DNA is twisted up like a telephone cord. Plasmids are found only in bacteria, certain viruses, phages, in some eukaryotes such as yeast, and in the mitochondria of all cells.[10]

Molecular biologists can transfer DNA into another cell by plasmid vectors, a role that plasmids were originally designed to achieve. This is a normal means that bacteria use to exchange DNA, a process which confers to bacteria some of the advantages of sexual reproduction. One of the reasons plasmids are modified by researchers is so that they will express the genes that they insert in them to produce proteins such as insulin.

Plasmids vary enormously, and for a vector to function properly the correct one must be selected. For example, plasmid vector PDR3-22 has only about 4,000 base pairs and only one Bam H1 restriction cutting site. Since Bam H1 cuts **only** in one place, a new gene can easily be spliced in between these open ends. The open base pair's ends are called **'sticky',** and the added gene segment must have complementary 'sticky' ends in order to bind to the vector ends.

When the 'sticky' ends undergo hydrogen bonding, they do **not** form a stable molecule, and these bonds constantly form and break again. The bond often holds the two strands together long enough, though, for DNA ligase to cause the DNA **framework** to bond, forming a stable strand. DNA ligase is critical for DNA work. It catalyses the formation of phosphodiester bonds between neighbouring 3'-hydroxyl and 5'-phosphate ends in double-stranded DNA, and it can also repair nicks in duplex DNA.

This procedure allows us to add a new DNA section to the plasmid and then use DNA ligase to tie together the sugar phosphate chain and repair any nicks. Ester links form by dehydration synthesis resulting in the **loss** of one molecule of water, thus the bond is called a condensation reaction. These techniques are possible only because the complexity of the cell system is understood sufficiently to enable us to exploit the existing mechanism, somewhat like an auto enthusiast can trade parts between cars to produce a modified automobile.

Splicing foreign DNA into a host cell is called transformation if bacterial cells are used, and transvection if mammal cells are used.[11] In transformation, plasmid DNA enters a bacterial cell and multiplies along with it. The researcher can readily determine if the new gene is spliced in by incorporating a gene for tetracycline resistance in the

segment. If the bacteria are grown in a petri dish with tetracycline, **only** those that are expressing the new genes in the plasmids will survive (see later).[12]

IMPROVING VECTOR ACCEPTANCE

Usually at least 10^8 to 10^9 bacteria are required for transformation to be successful because only a small percentage of bacteria will successfully take up and express the new gene. The number of transformations can be increased by suspending cells in a cooled calcium or rubidium chloride water solution, then subjecting them to a brief **heat shock,** usually around 42°C. This process causes improved 'competence', or a greater ability to take up the new plasmid.

Another method to make cells competent is to treat the vector with divalent ions such as Mg^{++}, Mn^{++} or Ba^{++} which are called transformation buffers. This will cause up to 10 per cent of all viable host cells to take up foreign plasmids. This method aids the uptake by causing the bacteria's outer membranes to become more porous, allowing more vectors to enter the bacteria.

The electroporation method of increasing cell competence requires mixing the DNA and bacteria in a cuvette, then passing high voltage electricity through the cuvette for a fraction of a second. This causes many tiny self-sealing holes to form in the bacterial cell membranes, causing improved competence.[13]

Another technique involves literally shooting the genes into the cells on tiny metal projectiles, a method appropriately called biolistics.

LAMBDA VECTORS

Use of a bacteriophage lambda (l) as a vector to chauffeur genes into bacteria is a process called lambda cloning. **Much longer** recombinant DNA lengths (rDNA) can be spliced into plasmids with this technique. The bacteriophage infects its host by mounting the bacterium, then inserting its DNA with a needle-like structure. After viral reproduction, the cell eventually lyses so that the viral offspring can infect other cells. We can normally splice from 8 to 10 kb of new DNA into a plasmid, but can fit up to 15 to 20 kb in a l phage DNA vector. Since a region in the middle of lambda's DNA is not necessary for replication, it can be replaced with the sequence to be cloned. For this reason, a lambda vector can incorporate DNA as long as approximately 75 per cent to 105 per cent of its genome length.

This method requires first isolating a DNA fragment of appropriate size, then ligating the DNA into the bacteriophage (all available in kits) and assembling the parts *in vitro*. The plasmid is first cut with a restriction enzyme, then the alkaline phosphatase de-phosphorylates the 5' phosphorylated ends of DNA or RNA. This prevents the de-phosphorylated vector DNA from re-ligating back to itself, thus closing up and preventing the insertion of the genes prior to the insertion of DNA fragments. This in turn will dramatically enrich the transformed *E. coli* with the plasmid containing the insert.

All plasmids have an origin of replication or initiation site, a starting point where a replicon (a unit which initiates replication) is located, and a replication termination point at which replication stops. Often we must use **different** replicons for a single bacteria batch — and can use a system called pUC (puck) if a large number of copies are needed. If we use the **same** kind of replicon for two inserts in the plasmid, the one that is **larger** replicates more **slowly** and eventually is lost from the cell because the two plasmids will compete for a limited number of replication factors.

The most versatile expression plasmids are those that have translational fusions, base pairs spliced into the plasmid to facilitate translation. There are two forms of expression plasmids that are utilised to overproduce proteins in *E. coli,* those that generate transcriptional and translational fusions. Transcriptional fusion plasmids utilise a strong regulatable promoter and the endogenous translational signals (ribosome binding site and initiator methionine) of the gene of interest. Translational fusions utilise a strong regulatable promoter, the ribosome binding site, initiator methionine and usually several additional amino acids from another gene. Transcriptional fusions have the advantage that the over-expressed protein is produced in its natural state, while proteins expressed as translational fusions contain a short foreign amino acid sequence 'tag' that is often utilised to purify the protein. These plasmids usually contain convenient cloning sites where a gene of interest can be inserted in the correct reading frame between an ATG base triplet (the translation initiation codon) and the coding sequence of the fusion gene.[14]

Some bacteriophage types such as a two-stage life cycle bacteriophage have what is called an F1 origin of replication. In the cell, Fl bacteriophage replicates itself as a double-stranded DNA, but when a virus package travels to the outside of the cell, it extrudes a single strand of DNA which is packaged in a protein coat. For multiple cloning site DNA, a series of **unique** endonucleases is used to make several cuts, each at a different restriction site. The fragment of interest, which also has the proper complementary set of restriction sites, is then ligated into the plasmid.

The researcher must also clone an expression vector into the plasmid. The expression vector must include the correct control signals, including a transcription promoter to cause an mRNA copy to be made. A proper fusion protein is necessary to fuse prokaryotic to eukaryotic genes because bacteria will express a high level of the protein **only** if prokaryotic recognisable amino acid sequences are at the **start** of the new gene. Then downstream,.a leader sequence must be added to cause the translation machinery to synthesise protein. This element is often referred to as a shine-delgarno sequence which is essentially a purine-rich (G, A bases) unit that serves as a ribosome binding site. It

is not understood exactly how the non-coding leader sequence functions, but it is a complex and accurate system.

This structure helps to position the RNA in the ribosome so that translation starts at the correct position — a goal achieved by certain base pairs on the 3' end of the ribosomal RNA (the RNA which is used in the ribosome, a machine used to help synthesise protein according to the mRNA master code).

SELECTING BACTERIA THAT WERE SUCCESSFULLY TRANSFECTED

Bacteria and other systems such as yeast are used for plasmid transformation so that the plasmid containing a DNA insert can be selected from the many thousands that do not contain the insert. The bacteria are also an easy way to make large quantities of plasmid DNA so that it, in turn, can be further manipulated by a researcher wielding other molecular biology tools. After cloning, a colony of bacteria is then grown, and instead of individual isolated colonies on an agar plate, researchers usually produce a lawn with **large areas** infected with bacteriophage.

To select bacteria that were successfully transformed, a drug resistance gene is included, such as for hygromycin, so that all of the cells will die **except** those that express the inserted genes. The non-transformed bacteria die producing holes in the 'lawn', and the remaining bacteria, called plaques, contain the clones of interest which can be selected out and grown on another petri dish to produce a population of transformed bacteria. Other ways of selecting the bacteria that were successfully transfected include use of the DNA code for B-lactamase, which degrades ampicillin, on the plasmid. If ampicillin is included in the medium, then only those bacteria with the enzyme will be able to grow. This effectively selects the few bacteria in a million that actually contain the plasmid of interest. Using antiobiotics in growth media together with a complementary antibiotic degrading enzyme is an extremely powerful and economical method of selecting only those bacteria that contain the plasmid of interest.[15]

Other enzymes can be encoded on the plasmid to select only those bacteria that contain a plasmid with the DNA insert present. This can be done, for example, by encoding a cell-death gene on the plasmid. This functions because only if the cell-death gene is disrupted by a DNA insert will the plasmid-containing bacteria survive.

Molecular biologists have developed many techniques so that it is increasingly easier to generate, isolate and characterise large amounts of protein. Bacterial or eukaryotic expression systems can now be made to produce the protein of interest at levels up to 50 per cent of the total cellular protein.[16] Eukaryotic proteins often require eukaryotic expression systems so that active eukaryotic enzymes which carry out the required post-translational modifications are produced. However, many proteins can be efficiently produced in *E. coli*, which is a simpler system.

THE ISOLATION OF DNA

The next step is to verify both the presence and the size of the cDNA inserted into the plasmid by growing a cell culture containing the vector. Then, the plasmid or other vector must be purified (separated) and the plasmid must be digested with restriction enzymes. The cut sections are run on a gel to determine fragment sizes and to verify that the expected cut sites and fragment sizes exist in the sample.

The DNA of interest can also be 'isolated' by cutting out the section of interest by restriction enzymes. These DNA fragments can then be ligated into an appropriate virus or other vector that carries the DNA into cells.

The above steps illustrate how much reliance is placed upon naturally existing mechanisms, including enzymes, polymerase and ligase systems, which illustrates that humans are simply manipulating the tools which had already been designed and built by the Creator.

A group of techniques called mapping are used to determine specifically where the genes of interest are on the chromosomal DNA. One of these techniques, called chromosomal walking, uses probes that are made from the ends of the cloned DNA fragment. These can be used to identify clones carrying DNA which was originally adjacent to this fragment on the chromosome. The probe could be radio-labelled, and if it adheres to a segment, it is known that the chains of interest are located on that segment. Another technique called chromosomal jumping uses a library of probes that can identify clones carrying the DNA which was originally in the DNA some distance away from the fragment of interest. Another technique is the PCR-base procedures, which locate sequences that are surrounded (bracketed) by known pairs of oligodeoxynucleotide primers.

COMMON DNA ANALYSIS TECHNIQUES

DNA length can be used to determine if a specific gene exists in a DNA sample if a restriction enzyme is known to cut the gene. If a cut is made, the gene of interest is present and two smaller DNA segments will result. If the gene is not present, a cut will not result and one long strand will still exist. One method that uses this technique is called restriction fragment length polymorphism (RFLP) analysis and is used to test for diseases and determine paternity. It can also help to identify criminals. Conversely, the technique has been instrumental in proving that hundreds of convicted rapists and others convicted of criminal actions were innocent.

In sickle cell anaemia, a mutation causes glutamic acid to replace valine, a change that can be determined by restriction enzyme analysis because it will cut **only** if the code for valine exists.[17] Since the DNA will be cut in normal persons, the **length** of the DNA fragment in a sickle cell anaemia patient will vary from the normal. It can be determined if a cut occurred at this critical junction because

shorter DNA segments migrate faster in electrophoresis.

Electrophoresis is a major method used to separate DNA strands, specifically and primarily by their length. When electrical current is applied, DNA's outer **negative** charge causes it to be pulled toward the positive end of a porous agarose or polyacrylamide gel medium. Agarose is usually used to separate large DNA molecules, while polyacrylamide is used for the separation of small DNA molecules and proteins.[18] Pore size is controlled by the concentration of agarose or polyacrylamide. The larger the pore size, the lower the pore concentration.

The major source of DNA's negative charge is the phosphate group in the polymer chain. After the nucleotides bind, DNA still possesses one ionisable hydrogen (H^+) atom, and therefore is a nucleic **acid,** but is ionised **only** in an aqueous solution of sufficiently high pH. A buffer is needed to ensure that the gel will effectively conduct electricity and not change the charge on DNA. The largest fragments remain close to the front where the DNA is loaded in a well, and the smallest fragments move to the other end of the gel, thus separating them by length. The size separation occurs because the shorter DNA lengths work their way through the gel pores faster than the larger ones.

This system is so sensitive that it can separate DNA fragments by a difference of only **one** nucleotide in 500. To help the researcher see the DNA, ethidium bromide or another stain is used in the loading gel so that the DNA fluoresces when exposed to ultraviolet light. The size of the DNA fragments can be determined by running what is called a **latter** with the sample. A latter is a set of DNA of known lengths, usually 123, 246, 492, 984, etc. base pairs long. By comparing the strand's run with these standards, their approximate size can be estimated.

If **several** forms of the same gene exist in an animal's genome, each one coding for the same protein, they are called polymorphic forms. Two types of polymorphic forms exist, the **sequence** form, a variation of the base **code,** and the **length** form, a variation of the **number of copies** of a repeating region. Instead of three copies of a repeat as is normal, seven or more may exist.[19] This information is commonly used to determine paternity. First, the researchers evaluate the mother's and child's genes, and then compare the results with the possible father's. Humans have diploid genes (two copies of each gene) because we obtain one from **each** parent.

An RFLP test is used to compare a set of base sequences to determine, for example, if all the selected genes can be accounted for by the mother and a specific male. Each row of the gel is used for a different comparison (such as the mother, the child and the alleged father). The child is **heterozygous** because he or she normally has genes from **both** parents. Thus, a different restriction map results from these differences in nucleotide sequence because differences in nucleotide sequence result in differing sites for restriction nuclease activities. Since any two individuals have an estimated more than one million base pairs that differ, this technique is useful for crime work to produce what are now called **DNA fingerprints.**[20] Usually only a tiny fraction of the potential variable sequence must be evaluated to prove it is highly likely that two samples do or do not match.

Electrophoresis can also be used to separate proteins by their size, shape and charge. Polyacrylamide gel electrophoresis (PAGE) is usually used. For DNA analysis PAGE is used under continuous conditions (one gel density, one buffer, and one pH), but for the analysis of proteins, discontinuous conditions (different gel densities, buffers, and/or pH's within the same gel) are usually used. Discontinuous gels can produce sharp, highly concentrated zones of material and make possible the separation of mixtures of highly similar substances.[21]

SOUTHERN, NORTHERN AND WESTERN BLOTTING

Another common DNA analysis technique called Southern blotting was developed in the 1970s by E. M. Southern to determine if **specific DNA sequences** are present in a gene. After restriction nucleases cut DNA into a series of fragments, the fragments are then separated by agarose gel electrophoresis according to their length, as described above. The DNA is then denatured by an alkali, such as sodium hydroxide (NaOH), to separate the DNA strands. The strands are then transferred by capillary action to a piece of nitrocellulose filter paper. A radioisotope-labelled single-stranded DNA probe that will hybridise (bond) only to **select** strands of DNA is then used to tag them.

The gel is then exposed to X-ray film to obtain a permanent black and white image called an autorad. An autorad can be produced because when the DNA probe is made, radioactive isotopes are used to tag them. Usually ^{32}P or ^{35}S radioactive isotopes are used for autoradiography, and exposure requires several hours. Since ^{35}S is a weak beta emitter, if only a few DNA strands are present, it may require weeks to sufficiently expose the x-ray film. One can in this way determine if the DNA of interest is present and the approximate length of the identified section.

If RNA is separated, the above method is called a northern blot. Northern blotting also uses an agarose gel and the same process as above, except that the alkaline denaturing step is not necessary because RNA is already a single strand. A western blot is used to separate protein electrophoretically, which is then transferred to blotting paper so that it can bind to it covalently. The proteins of interest are then located with a radioactive probe in a similar way to that used for southern blotting.

SEPARATING DIFFERENT TYPES OF DNA

One method used to separate DNA types is by buoyant density differences. G-C rich strands are **more** dense than T-A, because G-C has three hydrogen bonds and T-A only two. Consequently, differences in the A-T and G-C ratio in a DNA section allow effective separation. Density differences can also be used to separate **plasmid DNA** from the nucleus (chromosomal) type of DNA, because plasmid DNA is often supercoiled and is thus more dense. One method, caesium chloride (CsCl) separation, can be used to isolate both nuclear and plasmid DNA. **Satellite DNA** is a DNA fragment which has a markedly different buoyant density due to either higher or lower A-T residue level. It is either much more or less dense than the main band, and consequently forms a density gradient when placed in a caesium chloride solution.

Separation of duplex DNA and single-stranded DNA can also be achieved by exploiting viscosity differences of the two. DNA can be denatured by enzymes, temperature, or pH changes (or by alcohols, ketones, urea or amides), which break the hydrogen bonds between the base pairs, separating the two strands. Single-stranded DNA is **more** flexible than double-stranded DNA, and consequently is **more** viscous because it is usually more tangled up.

Renaturation is the process of separating duplex DNA strands and then, if properly aligned, causing them to rebind if **they are complementary.** If DNA and RNA strands that complement each other are hydrogen bonded together, the process is called hybridisation. Renaturation involves shearing or 'digesting' DNA with an enzyme that breaks the hydrogen bonds between the base pairs, then heating them so that they separate, and last, cooling them to cause reannealing (binding). This can be achieved in the lab by first heating the DNA up to 90°C to denature or 'melt' it, then **dropping** the temperature back to 40°C, and last, warming the solution back up so that the fragments will collide more frequently so as to increase the likelihood of bonding. If a collision occurs between complementary strands, they will 'zip up' and the two strands will again pair up together (reassociate) and form hydrogen bonds.[22]

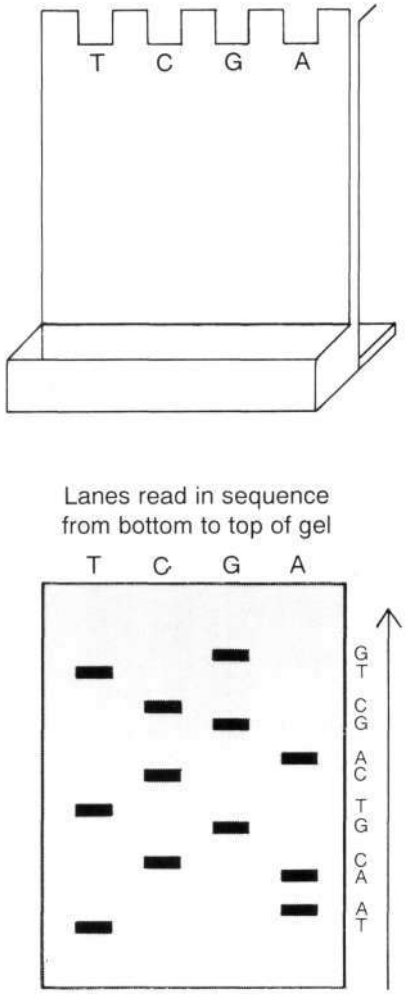

Figure 1. *Autoradiogram dideoxy technique using poly-acrylamine gei for 'reading' the letter sequence in DNA.*

THE PROCESS OF BASE SEQUENCING

A Common method of sequencing — determining the order of every base pair in DNA or RNA — is the chain termination method called dideoxy sequencing, developed by Sanger.[23] To sequence DNA in the lab by this technique requires a master DNA copy and the following:

(1) Magnesium ion (Mg^{++}) which is a co-factor for DNA polymerase catalysed linkage.
(2) The nucleotide precursors needed to construct the four bases (dGTP, dTTP, dATP, dCTP). This group of four bases as a set is called dNTP (deoxynucleotide triphosphates). Each of these bases are high in energy. Readers would be familiar with ATP, which is an analogue of dATP.
(3) Using DNA polymerase, and single-stranded DNA, a copy is made which stops **randomly** along the length of the DNA being copied. This is achieved by adding a small amount of dideoxynucleotide (ddNTPs) which randomly terminate the DNA polymerase chain-building reaction as it proceeds along the length of the DNA being sequenced. It does this because the dideoxynucleotide lacks a 3'-OH in the molecule, preventing further synthesis of the chain.

The DNA is divided into four aliquots, and the first is placed with the nucleotides so it stops copying on an adenine, the second so it stops on a cytosine, the third on a guanine, and the fourth on a thymine. The four DNA samples are then run on a polyaerylamide gel. The sequence can then be read because the separation by size is so sensitive that one nucleotide difference can be discerned. The DNA strand that is farthest away from the point of origin on the gel (the well) is the smallest. If this happens to be in the T-column we know the first letter of the sequence is T (see Figure 1).

Scientists have now sequenced several complete human chromosomes and also the complete genome of a few organisms such as yeast. The first complete genetic sequence was of a bacterium called *Haemophilus influenzae.*[24]

By sequencing the DNA of the Y chromosome from 38 men all over the world, the researchers concluded that no variation existed for the genes that they examined —confounding evolutionists and forcing the conclusion that all men are descendants from a man who lived

relatively recently.[25] Called the **Adam hypothesis,** it is one of hundreds of examples of how molecular biology has eloquently supported the creationist worldview.

SOME PHILOSOPHICAL CONCLUSIONS

The above examples of DNA analysis and recombinant technology effectively illustrate that the processes used are not unlike human manipulation of animals and plants for food or clothing. Humans are not creating life by use of these techniques, nor are they significantly altering biological or biochemical aspects of life. They are only exploiting for their own purposes the enormous complexity which **already exists** in the natural world. Although this technology is far more complex than, for example, using breeding, nutrition, or drugs and hormones to modify animals or plants for human purposes, the processes are significantly similar.

Most of the tools that researchers utilise already exist in nature, including restriction enzymes, plasmid vectors and ligation enzymes. A comparable situation is a person exploiting a tool shop he inherited, and although the tools may at times be altered to the machinist's advantage, the machinist did not create the machine shop or its contents; he simply utilised existing tools and procedures to modify existing materials.

Humans are only using the tools that God already created; tools that come from nature which are so extremely complex that only an omniscient and omnipotent God could be responsible for their creation. Random chance has no role in the creation of beautifully-precise technologically-advanced cellular machines such as the enzymes that have been described here. Biotechnology, probably more than any other field of study, has affirmed the concept of intelligent design. Every new discovery adds weight to the argument that an incredible intelligence was behind the creation of life in all its basic forms.

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