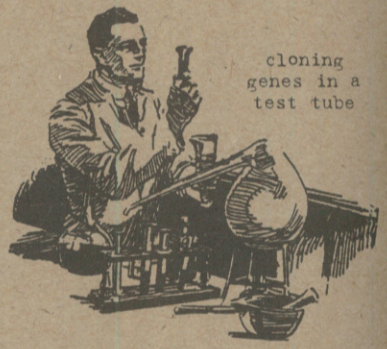
CHAPTER 4

USING OUR KNOWLEDGE



cloning genes in a test tube

*** Over the last few years all sorts of new technological tricks have emerged in molecular genetics. One of the most significant of these has been the Polymerase Chain Reaction (PCR). PCR is the geneticist's equivalent of the photocopier. Starting with just a few molecules of DNA, it can generate literally billions of copies of a specific DNA sequence in a few short hours.**

Kary Mullis

Voyage of Discovery

PCR was invented by American geneticist Kary Mullis, who was awarded a Nobel prize for his work. He claimed the idea came to him after a drug-induced hallucinatory trip into a DNA molecule!

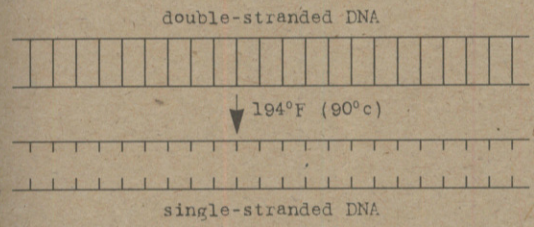
PCR AND PRIMERS

* ***PCR is a quick and simple way of cloning genes in a test tube***, without the need for bacteria, and has replaced conventional cloning techniques in many areas of molecular genetics. It does have its limitations, however: it can only be used when you already have some sequence information on the bit of DNA you wish to clone. Otherwise, it's back to bacteria.

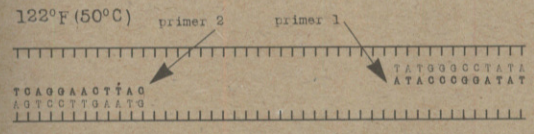
* Every PCR reaction requires a pair of DNA PRIMERS. These are short stretches of single-stranded DNA that are complementary to the DNA sequences flanking the gene which is to be copied. These primers go into the ubiquitous test tube along with a sample of DNA, generous quantities of each of the four nucleotides (A, G, C, and T), and some DNA polymerase enzyme.

THREE EASY STEPS

* The PCR reaction consists of a cycle of three steps repeated again and again. Each step lasts about a minute. In the first step, the reaction mixture is heated up to about 194°F (90°C), which separates the double-stranded DNA into two single strands.



* Although the primers are sitting in the test tube, they cannot bind to the DNA at such a high temperature. So in the next step, the temperature is lowered to around 122°F (50°C), which allows them to attach themselves to the complementary sequences on each of the single strands.



* In the final step, the temperature is increased to about 162°F (72°C). At this temperature, the DNA polymerase begins to synthesize new DNA strands, starting from each of the two primer sequences. Repeated cycles of the three steps produce a doubling of the DNA molecules each time. After about 30 cycles, the number of DNA copies will have increased about a billion times.

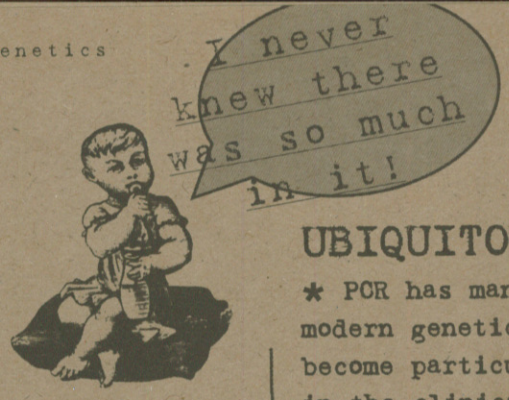
Thermocycler

PCR reactions are performed in a thermocycler—a machine that can rapidly shift between temperatures in a programmed order.

Hot Stuff

Ordinary DNA polymerase won't work at the high temperatures involved in the PCR reaction, so a special heat-resistant variety is required. This is derived from the bacterium ***Thermus aquaticus****, which lives in hot springs at temperatures of up to 194°F (90°C).*

the right temperature is critical



UBIQUITOUS PCR

*** PCR has many applications in modern genetics—but it has become particularly important in the clinical diagnosis of genetic disease and in the field of forensic science.**

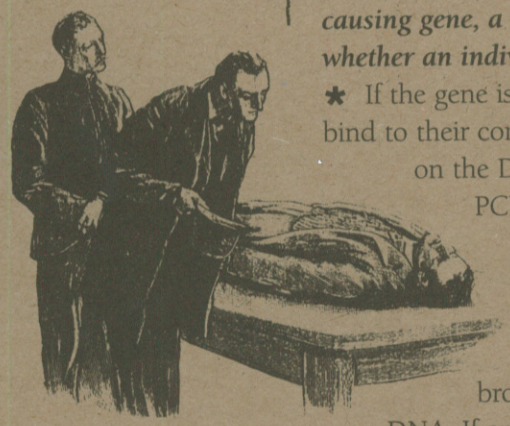
Effective range of PCR

PCR works best on segments of DNA that are longer than 100 base pairs and shorter than about 3,000 base pairs. The technique can be used on as little as a picogram of starting DNA—i.e. 0.000000000001 grams of DNA!

GENETIC SCREENING

* It is now common practice to screen the genetic recipes of prospective parents and fetuses for disease-causing genes, particularly when there is a family history of an inherited disorder. This is possible thanks to PCR. ***By using primers specific only to sequences found in the disease-causing gene, a PCR test can reveal whether an individual carries the gene.***

* If the gene is present, the primers will bind to their complementary sequences on the DNA molecule, and the PCR reaction will produce millions of copies of the defective gene. These are detected by electrophoresis and ethidium-bromide staining of the DNA. If an individual does not carry the gene, the primers will have no complementary sequence to bind onto, giving a blank result.



inherited disorders may soon be a thing of the past

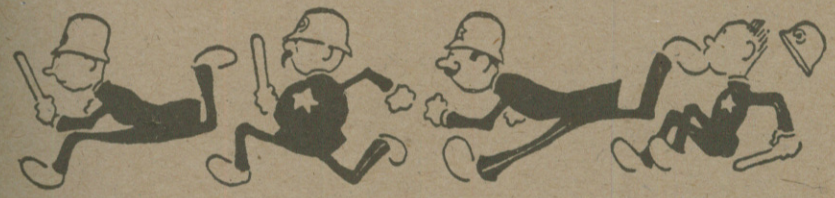
a single cell left behind can incriminate

DIAGNOSIS

*** PCR is used in a similar way as a diagnostic test for the presence of disease-causing organisms such as the HIV virus and *Mycobacterium*, the bacterium that causes tuberculosis.** In an HIV test, DNA is extracted from a small sample of the patient's blood. Then a PCR reaction is carried out using primers specific to sequences found only in the genes of the HIV virus. If the primers do not find a sequence to bind onto, the virus cannot be present.

Criminal copies

One of PCR's great strengths is that it works with even the smallest amount of DNA. For this reason, it has become an invaluable tool in forensic science. Crime scenes are full of potential genetic clues. Criminals often unwittingly leave behind small amounts of hair, skin, blood, and saliva. All these bodily tissues are made up of cells that contain copies of the criminal's genetic recipe. Although DNA can be extracted from a single hair follicle, the amount obtained is so minute as to be apparently undetectable—that is, until PCR is used to produce sufficient gene copies for a detailed genetic analysis.



forensic science has made leaps and bounds since PCR was invented

women are told about genetic risks before having a baby

Lethal genes

Each one of us carries about five or six recessive genes in our genetic recipes that would kill us if we had two copies instead of one. However, the appearance of such lethal recipes in a child depends on two carriers of the same gene meeting and both passing their recessive gene on to their offspring.

KEY WORDS

CARRIER:
a person who carries a single copy of a recessive disease-causing gene

GENETIC COUNSELOR:
a counselor trained to educate prospective parents about genetic disease and the risks for their children

CARRIERS AND COUNSELORS

*** Nowadays PCR is used to test whether prospective parents are carriers for some of the more well-known and serious genetic diseases. The results of such a test can help couples make informed decisions about whether or not to have a child and the genetic risks involved.**

CYSTIC FIBROSIS

* Cystic fibrosis is caused by a defect in a protein whose job, in normal individuals, is to maintain a chemical equilibrium across the membranes of cells. People who suffer from it experience an abnormal build-up of thick mucus in their respiratory tract and digestive system. Apart from causing breathing and digestion problems, the mucus is a perfect breeding ground for many species of dangerous bacteria, so infections such as pneumonia are common.

HYPOTHETICALS

* Imagine a situation in which a couple are planning to have a child. Both are perfectly normal, but each of them comes from a family that has a history of cystic fibrosis. There is a strong possibility that the parents both carry a single copy of the recessive cystic fibrosis gene. In such instances, they are likely to be referred to a genetic counselor who is trained to educate people about all aspects of genetic testing, and to help them understand any potential risk of genetic disease for their children. In this example, the genetic counselor would almost certainly recommend a genetic test for the cystic fibrosis gene. Of course, the decision as to whether to have the test ultimately rests with the couple themselves.

THE TEST

* A small sample of blood or a simple mouth swab is taken from both people to collect some cells. The DNA is extracted from the cells, and a PCR test using primers specific to the cystic fibrosis gene will reveal whether or not either or both of them are carriers.

* If neither or only one of them is a carrier, then there is no chance that their child could inherit the disease. Because cystic fibrosis is a recessive disease, an individual must inherit two copies of the cystic fibrosis gene, one from each parent, to get the disease. If both parents are carriers, then their child would have a one in four chance of inheriting it.

Tay-Sachs syndrome

This is an extremely unpleasant recessive genetic disease that results in blindness, severe mental retardation, and death before the age of five. In the U.S., the disease is common among Ashkenazi Jews. Arranged marriages are practiced in some Ashkenazi communities, and a genetic test can determine whether partners are genetically suitable for one another.

information is the key

DECISIONS AND DILEMMAS

an impossible choice

*** Sometimes the decision about whether to be tested for a specific genetic disease can be an extremely difficult and traumatic one. This is particularly true of Huntingdon's disease, which does not become apparent until middle age. The results of such a test will not only reveal the possible fate of a child, but also the fate of the parent.**

HUNTINGDON'S DISEASE

* Huntingdon's disease is a dominant genetic disease. If you inherit a single copy of the Huntingdon's gene, then you will get the disease. But until middle age anyone carrying the Huntingdon's gene appears to be perfectly normal. At some time between the ages of 35 and 50 the gene becomes active and the symptoms of the disease begin to appear.

is it better to know or to remain ignorant?

The Tiresias Complex

The dilemma confronting someone faced with the option of a test for Huntingdon's disease has been termed the Tiresias complex. In Greek mythology, the seer Tiresias confronted Oedipus with the dilemma, "It is but sorrow to be wise when wisdom profits not."

Woody Guthrie

The great American folk singer Woody Guthrie (1912–67), who was a major influence on Bob Dylan, was just one among several members of his family to die of Huntingdon's disease.

* These symptoms make unpleasant reading: severe mental and physical deterioration, uncontrollable muscle spasms, personality changes, insanity, and, ultimately, death.

* The gene for Huntingdon's disease was cloned in 1993, and there is now a simple test available to diagnose whether a person carries the gene. But anyone who suspects they have the gene faces an awful dilemma. Do they have the test and reveal their fate? Or do they remain in the dark and trust their luck?

at present there is NO CURE for Huntingdon's disease

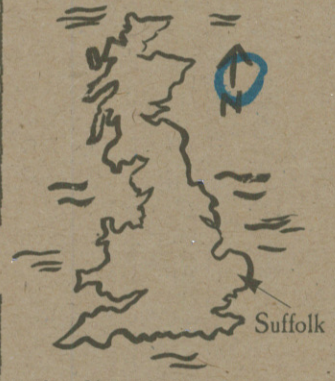
* Many people would go for the latter option. But if a person does have the disease and doesn't take the test, they may unknowingly pass the gene on to their children before the symptoms become apparent.

BUT...

* The only note of optimism in this nightmare of choices is that an early diagnosis of the Huntingdon's gene might contribute to the development of an effective treatment for the disease.

GEORGE HUNTINGTON

In 1872 **George Huntington** wrote about a hereditary defect "which exists so far as I know almost exclusively on the east end of Long Island." The ancestry of the disease was later traced to two brothers in Suffolk, England. It's ironic that the name of a disease caused by a mutation has itself gone through a mutational change. Due to a published misspelling in 1893, which went unnoticed, Huntington became Huntingdon and has been saddled with alternative spellings



the first known case of Huntingdon's was in Suffolk, England

EARLY-WARNING SYSTEM

*** Human embryos, as well as prospective parents, are now commonly screened for disease-causing genes and other genetic defects. If a genetic test reveals that child would have a short and painful life, then a mother may consider abortion as the most humane option. But not all genetic diseases are fatal, and early diagnosis of some diseases can increase the chances of successful treatment.**

PRENATAL SCREENING

Prenatal diagnosis is normally only offered to older mothers or those who have a family history of inherited disease. If it is likely that termination may be advisable, then clearly early testing is desirable. Unfortunately, neither amniocentesis nor chorionic villus sampling are ideal in this respect: cell samples cannot be taken until about 16 weeks and 10 weeks, respectively.

TAKING SAMPLES

* All kinds of information can be obtained about the genetic health of a developing embryo from just a small sample of its cells. The chromosomes can be looked at under a microscope to determine its sex, and to check whether there is the normal chromosome complement of 23 pairs.

prenatal screening gives early warning about genetic defects

About 50 genetic defects can be diagnosed by looking at the genes themselves (i.e. the DNA) or their protein products.

* There are two ways of obtaining embryonic cells from the mother's womb. In AMNIOCENTESIS, a sample of the amniotic fluid is taken from the mother's womb. This will contain cells shed from the embryo's skin tissue. In the alternative method, known as CHORIONIC VILLUS SAMPLING (CVS), a sample is taken from some of the embryonic cells that are destined to form part of the placenta.

SUCCESS STORY

* One of the most remarkable success stories of early diagnosis concerns the recessive disease PHENYLKETONURIA (PKU). Left untreated, PKU causes severe mental retardation. Babies born with the disease appear perfectly normal, but they are unable to produce an enzyme that metabolizes the amino acid phenylalanine. In the womb, the baby can survive on the mother's own enzyme—but after birth toxic chemicals build up in the baby's body, leading to irreversible damage to the central nervous system. If the disease is detected early enough, then the baby can be put on a special diet lacking phenylalanine; consequently no symptoms develop and the child grows up to be normal. Early screening for PKU has saved thousands of lives.

Tests

At the moment, tests exist for only a small percentage of the 5,000 or so known genetic diseases; and of those that can be detected, only a fraction can be successfully treated. Nevertheless, for the diseases that are treatable, early diagnosis can be important.

early diagnosis offers the best hope

BEFORE THE WOMB

*** There is now a new form of prenatal screening, called preimplantation diagnosis, which can benefit women who have a family history of a serious genetic disease and for whom abortion is either unacceptable or medically inadvisable.**

the sperm and egg are fused together in a test tube

TEST TUBE BABIES

About 10% of all couples experience problems in achieving conception.
In vitro fertilization (IVF) is just one of many infertility treatments that have been developed over the years. The first "test-tube baby" was born in 1978, in England. Since then, thousands more have been conceived.

thousands of babies have been conceived using IVF

TEST TUBE FUSION

* Preimplantation diagnosis screens an embryo for genetic defects before the embryo attaches itself to the mother's womb. This is only possible when the embryo has been produced by IN VITRO FERTILIZATION (IVF)—in other words, when sperm and egg have fused in a test tube, rather than in the mother's Fallopian tube.

FIRST TAKE A CELL

* The starting point for preimplantation diagnosis is a single cell taken from a three-day-old embryo. At this stage the embryo is still nothing more than a tiny ball of undifferentiated cells, and it is able to cope quite happily with the loss of one single cell. The removed cell can then be used to test for a whole range of genetic defects.

MORAL DEBATE

* If the genetic recipe of the cell is found to carry no genetic defects, then the embryo can be implanted into the mother's womb to continue its growth and development. If the genetic recipe reveals bad news, then the embryo will be discarded.

* The technique is morally unacceptable to some people, and in some countries it has even been outlawed.

FISHing is a new development in genetic screening

* In Britain, preimplantation diagnosis is often recommended for women who have already had several abortions because their embryos tested positive for a serious genetic disease. If there are medical risks associated with subsequent abortions, or if the woman is simply fatigued by the emotional trauma of repeated abortions, preimplantation diagnosis offers a solution.

FISHING FOR GENES

One of the latest ways of screening for defective genes is to use a technique known as **FISH (fluorescent in situ hybridization)**. Once a defective gene has been cloned, it is labeled with a fluorescent dye to make a DNA **probe**. After the probe has been added to the DNA of the cell being screened, a brief burst of heat makes all the DNA single-stranded. If the probe DNA sequence matches up with a complementary sequence in the genetic recipe of the cell, it will bind to it and show up brightly when viewed under a fluorescent microscope.

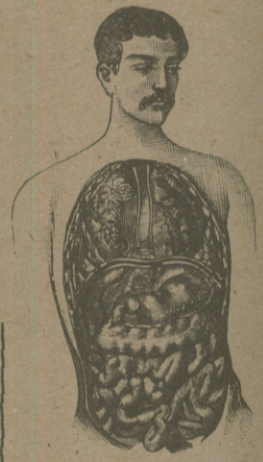
GROWTH FACTORIES

*** Biotechnology is big business. Agricultural, pharmaceutical, and medical companies are cashing in on the tools of the genetic revolution, in order to engineer new life forms with unique combinations of genes designed to suit human needs.**

the insulin factories

SWEET AND SOUR

* The first commercial applications of GENETIC ENGINEERING relied exclusively on bacteria, since the techniques of gene transfer in these organisms had already been well established. One of genetic engineering's earliest successes was in producing HUMAN INSULIN. Insulin is a protein hormone, produced in the pancreas, that controls the level of sugar in the blood. People who suffer from diabetes are unable to produce sufficient quantities of insulin. Lack of insulin means that blood sugar rises to dangerously high levels, leading to a variety of complications. Consequently, many diabetics have to inject the hormone into their bodies to keep their blood-sugar levels under control.



insulin is produced in the pancreas

Humulin

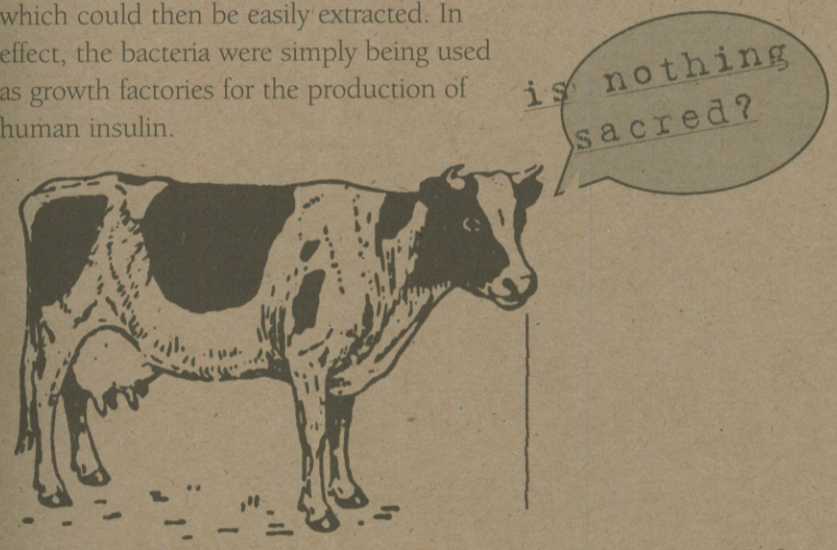
The first genetically engineered human insulin was marketed by the Eli Lilley Corporation under the trade name Humulin.

Hormones

A hormone is a substance, manufactured and secreted into the bloodstream by a gland, that regulates the functioning of another tissue or organ somewhere else in the body. Some, but not all, hormones are proteins.

CUT AND PASTE

* This injected insulin used to come from the pancreatic juices of cows. The only problem was that some diabetics had an allergic reaction to cow insulin. To get around this, SCIENTISTS IDENTIFIED THE HUMAN INSULIN GENE AND STUCK IT INTO THE GENETIC RECIPE OF BACTERIA. As the bacteria grew, they produced lots of the human protein, which could then be easily extracted. In effect, the bacteria were simply being used as growth factories for the production of human insulin.



Other hormones

A variety of other human hormones and proteins are now produced in a similar way to insulin. Hemophiliacs, for example, lack a normal working copy of the gene coding for Factor VIII, the protein that enables the blood to clot properly. But provided they receive regular injections of Factor VIII, hemophiliacs can live perfectly normal lives. Today, much of the commercially available Factor VIII comes from bacteria engineered with a working copy of the human Factor VIII gene.