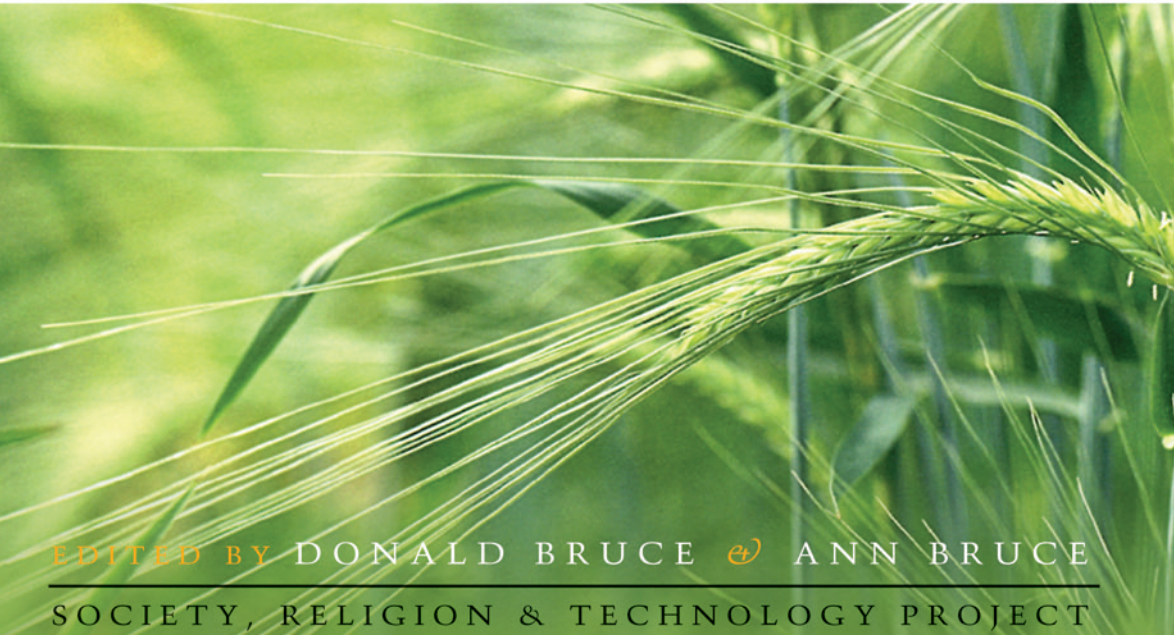
Engineering Genesis

The Ethics *of* Genetic Engineering *in Non-Human Species*



EDITED BY DONALD BRUCE & ANN BRUCE

SOCIETY, RELIGION & TECHNOLOGY PROJECT

ENGINEERING GENESIS
THE ETHICS OF GENETIC ENGINEERING IN NON-HUMAN SPECIES

Working Group of the
Society, Religion and Technology Project

Church of Scotland

edited by Donald and Ann Bruce

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'Dolly's eye view of the paparazzi – a social comment on how we treat the issues?'
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LIST OF ACRONYMS AND ABBREVIATIONS

AAT	alpha–1–antitrypsin
ACNFP	Advisory Committee on Novel Foods and Processes
ACRE	Advisory Committee on Releases to the Environment
BBSRC	Biotechnology and Biological Sciences Research Council
BSE	bovine spongiform encephalopathy
BST	bovine somatotrophin
Bt	*Bacillus thuringiensis*
CBI	Confederation of British Industry
DDT	dichloro-diphenyl-trichloro-ethane
DETR	Department of Environment, Transport and the Regions
EPA	Environmental Protection Act
EPO	European Patent Office
FAO	Food and Agriculture Organisation (United Nations)
FDA	Food and Drug Administration (US)
GATT	General Agreement on Tariffs and Trade
GMO	genetically modified organism
HIV	human immunodeficiency virus
IVEM	Institute for Virology and Environmental Microbiology
IVF	*in vitro* fertilisation
MAFF	Ministry of Agriculture, Fisheries and Food
MEP	Member of the European Parliament
NIABY	not in anybody's back yard
NIMBY	not in my back yard
NP	nuclear polyhedrosis virus
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
PG	polygalacturonase
PTO	Patent and Trademark Office
rBST	bovine somatotrophin artifically produced by recombinant DNA technology
RCEP	Royal Commission on Environmental Pollution
RNA	ribonucleic acid
SAGB	Senior Advisory Group on Biotechnology
SCRI	Scottish Crop Research Institute
SRT	Society, Religion and Technology Project (Church of Scotland)
TUC	Trades Union Congress
UPOV	Union for the Protection of Varieties (of Plants)

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This study is the product of a working group, and individual authors are identified only in the Case Studies in Chapter 2. Since it sets out to reflect a range of opinions, not every member of the group necessarily agrees with every point of view expressed in this book, but it is a product of the group as a whole.

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Dr Donald Bruce and Ann Bruce
Edinburgh
April 1998

INTRODUCTION

Genetic Engineering Has Arrived

Only within the moment of time represented by the present century has one species – man – acquired significant power to alter the nature of his world

Rachel Carson, *Silent Spring*,[1] p 231

Not many years ago, genetic engineering was just one academic area among many in the biological sciences. Within a surprisingly short period of time, the ability to isolate and transfer genes within and across different species has turned into one of the biggest growth areas in scientific research the world over, and one of the hottest areas of ethical debate. New discoveries are being announced almost by the week, and a technology is rapidly emerging whose products and applications are beginning to appear in our society. The prospect of genetically engineered food on our tables, crop plants in our fields and pigs' hearts in our bodies has either become reality, or has necessitated some serious ethical thinking about what should and should not become reality. The most spectacular example of the impact of biotechnology has been the unprecedented worldwide stir over the implications of cloning, following the breakthrough in sheep nuclear transfer at the Roslin Institute in Edinburgh. But it arose out of a piece of genetic engineering whose novelty in many ways encapsulates the revolution which is taking place.

Many people suffer from the debilitating lung disease emphysema. This is caused by a genetic defect, in which the lungs do not make enough of a protein called alpha–1–antitrypsin (AAT), which regulates the amount of an enzyme in the lung wall. The result is damage to the lung wall, which can eventually be fatal. Genetic engineering could offer at least three alternative methods for treating the disease, involving humans, animals or plants. In theory, it might be possible to go to the root of the problem using human gene therapy, attempting to incorporate enough of a non-defective gene into the lungs of the patient to increase the production of the protein. Such an approach is probably a long way off, but, as an alternative, genetic modification could be used to create novel ways of

producing the protein in another biological organism, on behalf of humans. It could then be purified and administered to patients as a conventional drug. Both animals and plants might offer this possibility.

In the 1980s, scientists at the Roslin Institute hit on the idea of producing AAT in the milk of sheep, by introducing a human gene into the sheep which 'codes for' this protein (that is, it sends a message which tells the body to produce it) in the mammary gland. The result was the sheep known as Tracy, and her progeny at PPL Therapeutics. Sheep produced in this way are now producing the protein for clinical trials. Roslin astonished the world in early 1997 when they announced they had cloned a sheep from the mammary gland cells of a ewe. Although Dolly became a famous celebrity overnight, she was really a sideline to the main research aim. This was to apply the technique of nuclear transfer to produce transgenic farm animals from a cell culture, something which had not hitherto been possible. This method happens also to produce cloned animals. A few months later Roslin and PPL Therapeutics produced the transgenic cloned sheep Polly from a cell line of genetically modified foetal tissue. This result may prove the precursor of many new possibilities for performing genetic modification in animals.

Meanwhile at the Scottish Crop Research Institute (SCRI) at Invergowrie, near Dundee, another breakthrough has opened up a way to produce vaccines, or therapeutic or industrial proteins, in plant tissue. This is achieved by genetically modifying a normal plant virus. The modified virus acts on the plant in such a way that it causes significant quantities of the relevant protein to be made in the leaves or other tissues. After harvesting the plant, the protein can be extracted and purified. This offers a straightforward way of producing a wide range of pharmaceutically useful proteins, and, perhaps, AAT.

Biotechnological Boom?

These examples of novel medical applications of genetic engineering represent the tip of an iceberg in a field that is growing at a bewildering rate. After the chemical revolution in the second half of the twentieth century, for many people biotechnology offers the next great hope in the quest for human security in food, resources and wealth. We are faced with an exponential growth in population, the diminution of agricultural land and other resources, and threats to the environment. Many politicians, scientists and industrialists look hopefully to genetic engineering to play a key role in feeding, resourcing and cleaning up past spillages and spoilages in the environment for a better future. In order to feed the

burgeoning populations of the less developed world in the first half of the next century, some see it as inevitable that we will need to use genetic techniques to derive new drought resistant or high productivity crops, or new generations of seeds which will be naturally resistant to fungi, viruses and pests, with reduced need for spraying with artificial chemicals. Marker genes are assisting conventional selective breeding methods in animals and plants to identify and control important traits for development.

Many see genetic engineering at the cutting edge of both future resources and the environmental crisis. Genetically engineered micro-organisms hold tremendous potential in dealing with sewage, pollution and oil spills, and in providing low toxicity alternatives in chemical production. More far reaching developments include the hope that genetically engineered crops might eventually become viable substitutes for oil as carbon based fuels, chemical feedstocks and perhaps even some minerals, which would be renewable and would not exacerbate global warming.

There are also prospects of large economic and employment benefits. Biotechnology has been big business for many years but, with such potential, genetic engineering could take it into a new phase. Most developed countries are investing heavily in research, and new gene based companies are springing up to harness the new research discoveries. In Europe, the European Union (EU) sees genetic engineering as a major source of future economic growth, worth many billion ecus, and potentially providing large numbers of highly skilled, high added value jobs.[2] The EU funds major research programmes seeking to enable its member states to maintain their market position against severe competition from the US and the Pacific Rim.

Biotechnological Bust?

It is easy to wax lyrical about the potential food, health, environmental and economic benefits from these new developments, but at the same time neglect their important ethical, social and spiritual implications. The rhetoric of their potential for some areas of human life needs to be balanced against the effects on other, equally vital, aspects of human society and also on the very animal and plant kingdoms to which we are looking to provide us with this bonanza. Should we be doing these sorts of things to our fellow creatures with whom we share the planet? How do we balance the harm we might do to them against the benefits to ourselves? And to what extent are applications to animals a short step to questionable uses in humans?

Do we know as much as we think we know? The ideals of science practised in humility and with due caution can easily be pushed aside by academic success and commercial prospects. Exaggerated claims can give the impression of having the technology more in our control than we actually do. The recent history of science based technology suggests that new developments seldom turn out to be quite as straightforward or utopian as their proponents and backers suggest. Are we putting ourselves unnecessarily at risk in proceeding too fast after the goals we can see, and turning a blind eye to the problems we would rather not see?

As we are now seeing with the motor car, once we develop social dependencies on a new technology, it becomes very difficult to change if unforeseen problems start to emerge later. It is pertinent to ask to what extent our society has, as it were, swallowed whole certain perspectives and assumptions that technological progress is the only possible way forward, without pausing to ask if there are better alternatives. Are we indeed, as some say, 'playing God', violating something which we should not be seeking to change in the natural order? Will it open up a Pandora's box which will cause misery and oppression of the poor, instead of the promised riches and blessings? Innovation requires social approval, and a wider acceptance of the challenges to lifestyle and values which it will bring. A society should always have the right to say 'yes' or 'no'.

Collision Course

Shortly after this book was published in November 1998, genetically modified food erupted in political and social controversy, largely through a sustained media campaign by a coalition of environmental and development NGOs, consumer groups and some sections of the media themselves. This rapidly polarised attitudes into two camps. Opponents view the prospect of genetic modification as an ideological mistake. Instead of addressing the radical question about how we produce our food, it carries the trend of chemically dependent industrial agriculture into foolhardy new realms, whose environmental and heath risks have not been properly studied. It puts even greater power in the unaccountable hands of multi-national corporations, and further impoverishes the poor of the world. Advocates see GMOs as a crucial option for future world food supplies, and stress their environmental and economic potential. They regard many objections as irrational fears stirred up by those opposed in principle to new technology, who harbour dangerously romantic notions of alternative 'organic' solutions and past ages that never were. Scientific and official circles stress the need to be more rational and

to educate the public out of needless fears. Fears of the unknown and unfamiliar undoubtedly play a part, but the public have also expressed a sharp reaction against having genetically modified food imposed upon them unlabelled and unsegregated, and are sceptical of Government assurances about food, after the BSE crisis. Belatedly there is official realisation that ethical issues and public misgivings must be taken seriously, and that proponents of biotechnology may also foster personal value judgements that are not always cool and rational.

Last, and by no means least, there is the spiritual dimension. Where do these developments leave us spiritually? If human life is more than material growth and scientific progress, we need to ask how the latter will affect our awareness and our relationship to God, our fellow human beings and the rest of the creation. The question of what we can do can only be truly answered having first asked what we should be.

About this Report and the Working Group

In December 1993, the Society, Religion and Technology (SRT) Project of the Board of National Mission of the Church of Scotland (see Appendix 4) set up a multidisciplinary working group to look at ethical issues in the genetic engineering of non-human species. Its members were chosen for their expertise in a variety of fields relevant to the issues. These included specialists in the genetics of animals, plants and micro-organisms, in animal welfare, developing world applications, issues of risk and public perception, sociology, the environment and ethics. With this spread of expertise and viewpoints, the group has examined many of the complex issues which genetic engineering is bringing to light. We have not sought to follow any one theory of moral philosophy, in a logical and worked-through set of arguments. Rather, we present the iterative process of a diverse group of people studying issues, where each has brought the insights of their different disciplines to bear.

Our aim has been to strike a balance between the extremes of optimism or pessimism in which these issues are often framed in the media, drawing on the varied insights which such a group brings. Our membership included those enthusiastic for and those sceptical of the technology, and others who approached it undecided. Our group represented a microcosm of the broader societal debate. While we are a set of articulate experts who are not a typical slice of society, each of us felt a lay person in at least some of the diverse fields we discussed. We have tried to write for a general, non-expert readership, giving enough technical content to understand the issues, but without undue detail and jargon!

The book is not necessarily meant to be read in strict page order. Some will want first to read the basic concepts of genetics and its potential applications in Chapter 1. Other may find it easier to begin with the case studies in Chapter 2, or go straight to the chapter that most interests them, and go back to case studies and technicalities later.

The study was initiated by the Church of Scotland, and while this book stems from a basic Christian theological motivation, our aim is much wider than the Christian community. This is reflected in the mixed composition and way of working of the group. Indeed, one of the most important factors of our study has been the process of discussion and debate which has been generated by the different perspectives, beliefs, disciplines and experiences of the group members. This has, we believe, provided a richness and balance to our study which makes it unique among other works in this growing field. We present this book not so much to declare our agreed positions – for we have often agreed to differ – but rather as the result of our process of learning together. For each it has proved a valuable and stimulating journey of understanding, stretching our minds and our hearts into unfamiliar territory, but usually with at least one of us who knew how to read the map at any given point on the trail.

It is an unfinished task, for several reasons. We could not possibly cover all the ground we would have liked, and had to be selective in our explorations. There is much uncharted territory, and probably one or two dragons we have missed. Secondly, we have already seen that the technology and the public debate are changing fast. By the time the text is read, some aspects will already have moved on. Tomorrow may well raise new issues, and some of the old ones may in time seem less relevant. But today, we do not have the benefit of this hindsight!

The study is also unfinished in a third and perhaps most important way. We have not written the last word on any of these issues. We have already found the book much appreciated by those directly engaged with these fields – scientists, students of genetics, agriculture, environment, medicine, ethics and theology, and also industrialists, environmental organisations and politicians. But we have written for a wider general audience, to whom we present our work. We offer guidance and insight to those puzzled by the complexity of the issues, those confused by claims and counter-claims of vested interests, and those seeking stimulating and well informed views. We offer these some directions for how to look at these issues for themselves. And we also present them as an continuing process, as an invitation for you, the reader, to join in an ongoing dialogue with us. Your thoughts may add to the continuing reflection process. So we invite you to reflect with us, and, if you will, to share your reactions and thoughts with us, as we have sought to share ours with you.

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1 EXPLAINING GENETIC ENGINEERING AND ITS USES

Shaping the Creation

In the book of Genesis, the ancient biblical account of beginnings, God invites human beings not simply to flourish and reproduce their own kind, but also to take control over and care for the Earth as one might work a garden, to name the animals, to search for minerals, to relax and enjoy 'the cool of the evening', and to do it all in a warm and reciprocal relationship with God.

Since flint axes, knives and arrowheads, humans have always practised technology. From the earliest times, they have adapted the natural world to serve their own ends. This is a feature of human living. At the most basic level, humans have found ways to channel and shape natural things and processes to meet the necessities of providing themselves with food, water, shelter, warmth, clothing and so on. This has included domesticating and using living creatures, both plants and animals. Having achieved a measure of security, our energies need not be exhausted just in ensuring day-to-day survival. A myriad of other possibilities open up before us – to inquire, to create culture, art and civilisation, and to do new things with the natural environment. As we learn to innovate, and especially as modern science leads us to an unprecedented understanding of the workings of the natural order, new devices, ideas and methods are discovered.

Technology always comes out of some social context. A new invention may have started off to solve a recognised public need, with general approval. In our own times science has become a complex specialist discipline, remote from ordinary people. It is now far more likely that the scientists surprise society with what they have discovered. Again, those with political or socioeconomic power may have commissioned the new development and then simply imposed it upon others. In whatever way a technology is assimilated, however, it in turn remoulds society in some way. Things are never quite the same afterwards. It shapes our horizons and expectations. Western society in particular has come to expect techno-

logical advances and a continuous, incremental rise in living standards, security and wealth.

Technology thus brings challenges to society. Often changes are only minor, but from time to time a new development poses radical questions to our assumptions and self-understanding, to lifestyles and habits, to social patterns, and to our ways of relating to each other and to the natural order. Genetic engineering is one such development. The ability to manipulate the biological world at its most fundamental levels – the heritable genetic code of the deoxyribonucleic acid (DNA) molecule and processes of the cell nucleus – ranks as one of the most important developments in technology of the twentieth century. Although in some ways it can be seen as part of a chain of developments from the technology of the past, it is a way of shaping creation that has begun to raise some of the most profound questions of our times.

In the first instance, it poses these questions for developed Western societies, like Scotland, whose fabric is reasonably stable and secure, and where people have the luxury of time to ponder such issues. But the implications and effects of genetic engineering are global. If survival depends on the next crop or the next meal or a drinkable water supply, then the ethics of applying new technology assumes a lesser significance.

It is in this context that this chapter sets out to explain something of what genetic engineering is, for those unfamiliar with it, and to illustrate something of the range of applications of gene technology to animals, plants and micro-organisms, by way of introduction to the 11 case studies. The theme of this book is how this world interacts with the world of ethics and values, whose concepts and terminology can seem equally unfamiliar to many scientists. Some of the basic tools and terms used in ethics will be explained in Chapter 3, as well as how these are applied to the fundamental issues which underlie genetic engineering. Appendix 3 will suggest some methods which readers may find helpful to assess the ethical issues for themselves.

AN INTRODUCTION TO GENETIC ENGINEERING CONCEPTS AND TECHNIQUES

What is Genetic Engineering?

Genetic engineering is a very broad term which covers a range of ways of manipulating the genetic material of an organism. It is also variously called gene manipulation, genetic manipulation, recombinant DNA technology, the new genetics, targeted genetics and, in humans only, gene therapy. In

popular thinking it is frequently confused with cloning, which is not at all the same. The cells of living organisms contain genetic material which regulates the processes of the organism. This genetic material consists mostly of the complex chemical known as DNA, although sometimes it involves the related chemical RNA (ribonucleic acid). Pieces of this genetic material form genes and it is the ability to identify and manipulate one or more of these genes which underlies genetic engineering. It is estimated that there are something like 100,000 genes in a mammal and about 80,000 in a plant. Genetic engineering can involve manipulating genes both within species or between species. The products are generally referred to as genetically modified organisms (GMOs), or transgenic organisms. Since the early 1970s, genetic engineering has developed very rapidly as a powerful new tool for both the biological research sciences and the biotechnology industries, and an increasing number of applications are being brought to market.

Selective Breeding

Human beings have in a sense been performing a type of genetic engineering in the form of selective or 'classical' breeding ever since we ceased being hunter-gatherers and settled down to domesticate animals and crop plants some 10,000 years ago. Breeding involves the enhancement of recognisable traits in an organism by selecting for reproduction the individuals or populations which best exhibit the desired trait. This occurs in micro-organisms such as bacteria and the yeasts used in brewing and baking as well as in plants and animals. Some selection has taken place in response to social whims and fashions such as the desire for particular colours in domestic animals and ornamental plants. In a production context however, 'improved' varieties and breeds are selected empirically to enhance key characteristics such as higher yield, better industrial performance (eg wheat for baking, barley for making whisky) or greater disease resistance.

The process of selective breeding requires a number of generations of the organism. Because of the nature of growing seasons or an animal's generation cycle time this means that the process is cyclical and usually slow. Many of the traits of interest are controlled by multiple genes, each of which has a small effect on the trait. The trait may also be affected by the environment in which the organism finds itself. It may become difficult to discern whether an animal has the desirable characteristic because of its genetic inheritance or because of the environment it has experienced. Making the necessary breeding judgements can be highly

sophisticated, using complex statistical analyses of the different traits, but it still ultimately relies on the outward appearance or the measurements of traits to indicate the genetic value of the organism.

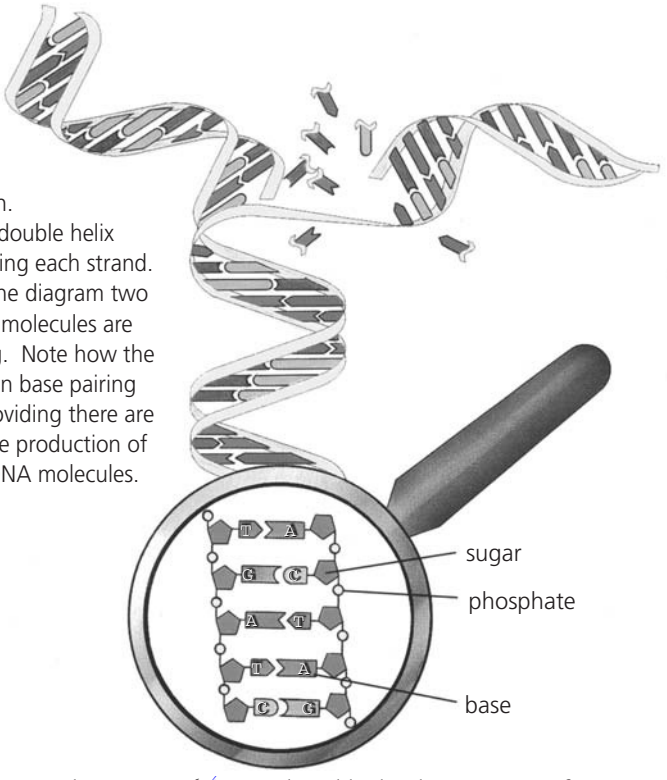
The above factors mean that selective breeding is by nature somewhat imprecise and unpredictable. Another important source of imprecision arises because natural sexual reproduction involves exchanging and mixing thousands of genes from each partner. The resulting offspring inherit an *almost* random mixture of the characteristics of both parents. The mixing is not completely random, hence some characteristics tend to be inherited together. When a breeder selects a crop plant or a cow for one particularly desirable trait, such as larger seeds or more milk, huge numbers of other genes are also selected. These genes confer a range of other traits, many of which are unknown or biologically neutral. Sometimes, however, genes for some undesirable characteristics such as predisposition to a disease may also be present in the selected progeny. A kind of halfway stage between classical breeding and genetic engineering is of increasing interest to animal breeders. This is called marker-assisted selection, and involves using genetic markers which are associated with one of the desired traits in order to identify the 'best' animals or plants to produce the next generation. Other genetic changes can be induced using chemicals or irradiation, as discussed in Case Study 2.

In contrast to classical breeding, genetic engineering now offers the capacity to add a single gene, or a small cluster of genes which control a new trait. This would be added to an already tried and tested variety of a given species. In some cases genes can also be deleted or have their function disabled. One major difference between this and classical breeding is that genetic engineering does not involve mixing the entire complement of genes between two individuals, but only the particular genes which have been identified. Genetic engineering should therefore be more rapid, precise and predictable when compared with selective breeding methods. The latter have jokingly, but not entirely unfairly, been summarized as: 'pick and cross two of the best, then hope for the best!' Genetic engineering is, however, still a relatively young science, and, for all its successes, it is not without its hit and miss elements.

The Principles of Genetic Manipulation

DNA

The fundamental chemical and biological discoveries which made genetic manipulation possible can be traced back to early studies on bacterial



DNA replication.
A parent DNA double helix unwinds exposing each strand. At the top of the diagram two daughter DNA molecules are shown forming. Note how the process relies on base pairing and results, providing there are no errors, in the production of two identical DNA molecules.

Source: Straughan and Reiss (1996)[4] reproduced by kind permission of BBSRC

Figure 1.1 *DNA Replication*

genetics in the 1940s, and to the discovery of the structure, and hence the modus operandi, of DNA (Figure 1.1) in 1953.[3]

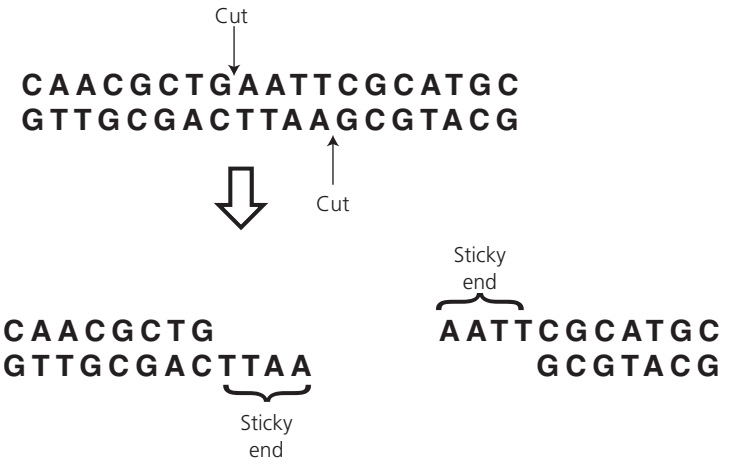
DNA is a biological polymer which contains all the essential chemical and developmental information which the cell needs to perform the biochemical processes of life. It does this by means of a complex chemical code, which consists of different combinations of a very large number of smaller chemical groups called bases, which are attached to an inert backbone forming the helical structure of the DNA molecule. There are only four types of base, which are abbreviated by the letters A, C, G and T. It is the sequence of these bases, in different orders and combinations along the backbone, which makes up the genetic information – the genetic code. Broadly speaking, each group of three bases forms the code for a single amino acid. A chain of these amino acids will lead to the production of a protein which will affect the biochemical processes in the

organism – just as letters form into words which convey to us particular meanings. Some of these bases act as punctuation marks, for example to identify the beginning and end of a protein. Other sequences of bases have functions which are not yet fully understood. The length of DNA which is required to produce a single protein is known as a gene. The gene is said to 'code for' that particular protein and the operation of the gene to produce the protein is known as the 'expression' of the gene. Further details of the structure of DNA are given in Appendix 2. RNA is similar to DNA in structure, but the chemistry of a sugar in the backbone and one of the bases is different. RNA plays several key roles in the production of a protein from the DNA code. In some organisms, such as viruses, RNA is the only form of genetic material present.

Gregor Mendel was a German monk whose study of pea plants in the nineteenth century identified the basic rules of genetics and inheritance. Work between 1940 and 1970 on the genetic material of common bacteria such as *Salmonella typhimurium* and *Escherichia coli* then provided a far more detailed operational definition of a gene and an understanding of how its expression could be regulated. Many elegant studies used mutant bacterial strains or exploited natural viruses of bacteria (known as bacteriophages) which can jump in and out of the bacterial chromosomes and move pieces of DNA around. The small size of their DNA, their rapid growth and the short time between each generation of bacteria were key factors in the effectiveness of these genetic studies.

Cutting, Pasting and Copying DNA

Genetic engineering only became feasible when a number of key discoveries had been made. These enabled DNA molecules first to be isolated from the originating organism, then 'cut and pasted' in defined ways, and introduced and integrated into the normal DNA of a recipient organism. Until the early 1970s, the only way to isolate a small piece of DNA was to shear the entire DNA from the chromosomes of a cell by mechanical force. Unfortunately, this only produced random fragments of DNA so that no two fragments were likely to contain the same sequence. The breakthrough came with the discovery of a family of enzymes which are part of a natural defence mechanism of bacteria and which are able to cut DNA at specific places. These enzymes are called restriction endonucleases and each of them recognises one particular short sequence of bases in the DNA and makes a precise cut at that point. One enzyme might recognise the sequence GAATTC, another AGTACT, for example. When any DNA is cut it always leaves the same pattern of bases where the cut was made,



The action of the restriction endonuclease EcoRI. This finds the DNA sequence GAATTC and then cuts the DNA between the G and A bases.

Source: Straughan and Reiss (1996)[5] reproduced by kind permission of BBSRC

Figure 1.2 *Cutting, Pasting and Copying DNA*

regardless of the organism or species. Thus, if DNA from one organism is cut by a particular restriction endonuclease, and the same enzyme is used to cut a fragment of DNA from another organism, the two fragments could be stuck together at that point (Figure 1.2). In this way, particular fragments of DNA from different sources can be made to link together. Further details of this process are described in Appendix 2. The product of this is called a recombinant genetic sequence, or recombinant DNA, and it is then multiplied many times over and introduced into bacteria, plants or animals.

The multiplication process is usually called cloning. One method of cloning involves the repeated copying of the base sequence of the recombinant DNA by means of a carrier, which is known as a vector. The vector can be a chromosome, plasmid or virus. A plasmid is a small closed loop of DNA which multiplies to very high numbers in a single bacterial cell. The new gene is attached to the vector, and when the vector goes about its normal business of replicating itself, the new gene is automatically copied too; see step 3 in Figure 1.3. Alternatively, a chemical method is used, known as the polymerase chain reaction (PCR). This uses an enzyme to make repeated copies of a section of DNA in a chemical solution.

Gene Sequencing

These techniques of cutting, pasting and cloning small, defined pieces of DNA enabled new and otherwise unobtainable combinations of one or more genes to be produced from any organism. They also permitted the rapid development of techniques for sequencing DNA – that is, to determine the sequence of bases in a given DNA molecule. Over the past 20 years, these methods have become routine and automated, and are capable of operation by robots in the large scale sequencing programmes like the human genome project and analogous projects on such organisms as yeast, rice and pigs. In their simplest form, the materials for manual DNA sequencing are available in convenient 'kit' form. In a single tube, with sub-microgram amounts of DNA in a few microlitres (a tiny drop) of reaction mixture, it is possible to obtain the sequence of 500 to 1000 bases of DNA in a few hours. To put this in context, in 1965 the first complete gene sequence was determined. This was of a single-stranded RNA of only about 80 bases from yeast. It took many years of work, many hundreds of research staff, several grams of pure RNA and won a Nobel prize!

Over the past 25 years, the techniques of cutting, pasting, cloning and sequencing DNA, of designing new gene sequences, and of making specific alterations to existing gene sequences, have all become routine tools in research and development in the biological sciences. Their application has unravelled details of complex structures and function within cells, tissues and whole organisms. It has greatly advanced our knowledge of microbial, plant and animal life both in their normal processes and in what happens during disease.

Transferring Genes

Techniques to transfer DNA have developed over the past 50 years. They began by exploiting knowledge of natural processes in bacteria where DNA molecules move between cells – the bacterial equivalent of mating. Viewed as a whole, DNA transfer methods today can be classified as either biological or mechanical. The former still rely largely on bacterial or viral DNA vectors, whereas the latter include all manner of devices from injections, guns and microbullets, to darts, sparks, abrasives, water and salt shocks!

In order to make the 'foreign' gene express itself in the new DNA background into which it has been introduced, a special sequence called a promoter is always added to the introduced gene. Promoters act as signals to determine when and where the gene is 'switched on' to produce the new protein. Plant gene promoters respond to external signals such as light/dark, drought stress, salinity, plant hormones, wounding stress, or

just by being part of a cell destined to become a root not a flower, or *vice versa*. In animals, promoters can respond to particular items in the diet and can determine where the foreign protein is produced. In Case Study 8 the gene which results in the production of human alpha–1–antitrypsin in sheep was linked to a promoter which caused the protein to be produced only in the mammary gland, whereas the sheep version of the protein is produced mainly in the liver (just as the human version is in humans).

Current Gene Transfer Technology for Micro-organisms

Genetically modified bacteria are in some cases produced in their own right, as in Case Study 1 or for example in pollution control applications. Their most frequent use is, however, as the means by which other genes are multiplied. During the routine cloning of any gene, even one destined for insertion into a plant or animal, it is most likely that the early steps involved multiplication of the DNA sequence in a bacterial plasmid. Once the DNA fragments have been joined together in a test tube, they are introduced into bacterial cells which have been rendered 'competent' to take up recombinant DNA by following a carefully controlled series of growth and salt/heat/cold shock treatments. These procedures appear empirical but necessary for efficient DNA entry. Special bacterial strains are available for these procedures. These strains require selective nutrients and growth media as they have been genetically debilitated and are incapable of surviving if they escaped into the 'wild' by accident. They are also unable to recognize the incoming DNA as foreign. To select only those bacterial cells which have taken up the foreign DNA, it is usual to also include an extra gene which confers resistance to a particular antibiotic. This antibiotic is then added to the growth medium, and the only bacteria which continue to grow are those which received the recombinant plasmid molecule attached to the antibiotic resistance gene. Other techniques for uptake of engineered DNA are described in Appendix 2.

Gene Transfer Technology for Plants

Gene transfer technology for plants involves methods to introduce foreign genes into the plant cells and methods to regenerate whole plants from the transgenic plant cells. Figure 1.3 illustrates the various steps in plant genetic engineering.

Methods of Introducing Foreign Genes into Plants

Experiments on the transformation of plant cells began in 1980 and the first genetically engineered plant (tobacco) was reported in the scientific journal *Nature* in 1983. The first successful method for DNA transfer into plant cells used a natural soil-inhabiting bacterium called *Agrobacterium tumefaciens* which infects wound sites on plant stems. In the wild, *A. tumefaciens* induces cankers or crown galls by producing plant hormones which promote uncontrolled cell growth. It does this by inserting a portion of its DNA into the plant. By adding the 'foreign' gene to *A. tumefaciens* it can 'hitch a ride' into the plant cells. Further details of the process are given in Appendix 2. This natural, and so far unique, DNA transfer mechanism is efficient. It was used by scientists in Belgium, Germany, Holland, the US and the UK in the early 1980s to transfer designer genes and antibiotic resistance marker genes into plant cells in culture, into the damaged cells at cut leaf surfaces, or into stem cuttings (see step 5 in Figure 1.3). The system was primarily appropriate for broad-leafed plants. For many years, however, there were technical problems in transferring foreign DNA into cereals, bulbs, legumes or grasses, which were either not normal hosts for *A. tumefaciens*, or which presented tissue culture and plant regeneration barriers.

To transfer and integrate foreign DNA to the cells of plants outside the host range of *A. tumefaciens*, mechanical means were invented. The most theatrical and now most widely used of these was the so-called biolistic method, using a particle gun, first made in 1987 at Cornell University in the US. The genetically engineered DNA is precipitated onto the surface of extremely small gold or tungsten particles (less than a millionth of a metre across). The particles are then placed on the blunt front end of a macro-projectile which is fired at a stopping plate with a small central hole using a blank cartridge or high pressure burst of helium gas. The macro-projectile hits the plate, but the gold or tungsten micro-projectiles keep moving and hit the plant leaf, root, stem, cell suspension, callus or whatever tissue is placed under the stopping plate. Those cells which get bombarded too violently die, while those on the outer perimeter of the blast do not receive enough DNA-coated gold or tungsten to become transformed. However, a ring of cells midway receives enough DNA at the correct speed and force to penetrate the thick plant cell walls, the delicate cell membranes and the nuclear membrane to enter the nucleus of the cell which contains the DNA. Once inside the nucleus the foreign DNA is somehow inserted into the plant chromosomal DNA. Numerous variations on the biolistic DNA transformation process have been tried and tested over the past ten years. Other less commonly used methods of gene transfer are described in Appendix 2.

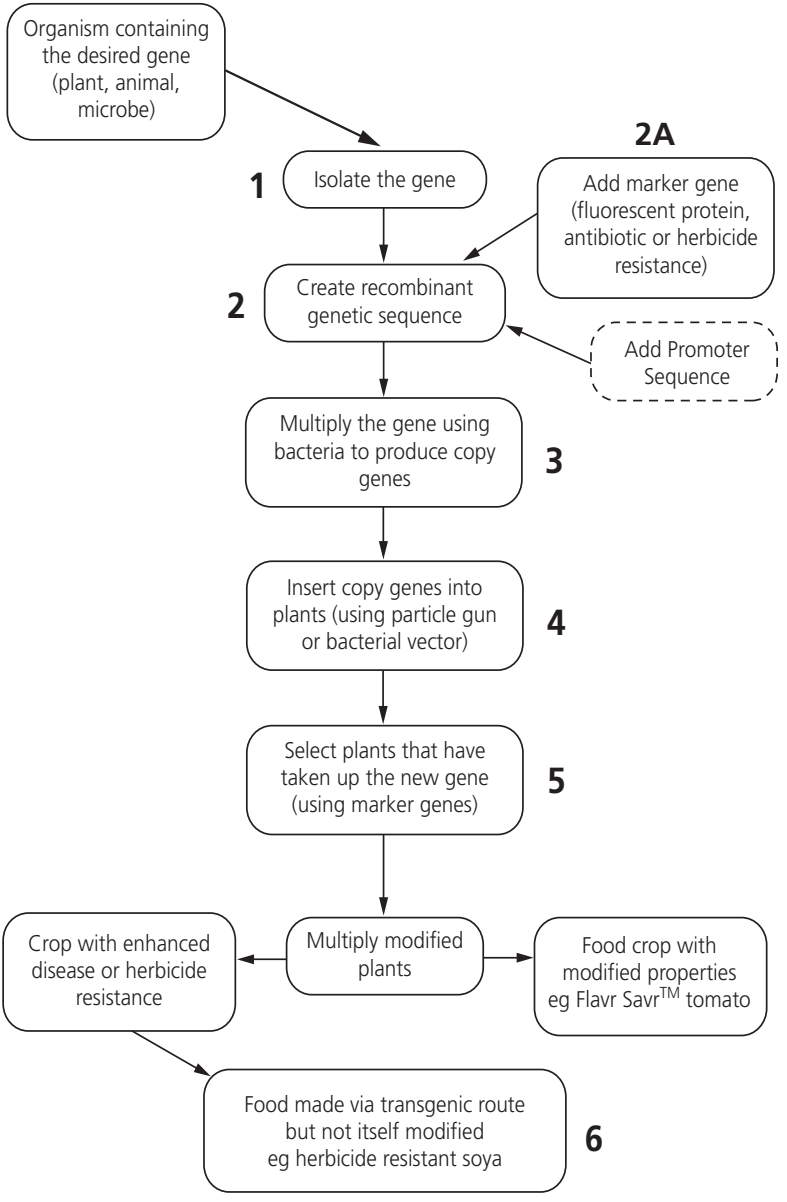


Figure 1.3 *Steps Involved in Plant Genetic Engineering*

The number of copies of foreign DNA inserted and the sites where this happens in the plant chromosomes are entirely random for a given cell. The foreign DNA may be inserted at several different sites and in several different chromosomes, but a single copy inserted at a single site is the most typical. Sometimes the DNA transfer and random integration processes place the foreign gene next to or even inside an essential plant gene sequence. This causes those plants to either to fail to grow, or to grow with some undesirable trait. Such abnormal lines are routinely discarded.

Attempts have been made to transfer more than one new gene into a plant at a time. This is important if complex biochemical pathways are required, or if multiple disease resistance is essential for a valuable but highly susceptible crop. In general, only single gene traits have been added to transgenic plants to date, but a number of ingenious strategies are now making it increasingly likely that multi-trait transgenic plants will be feasible. Methods which involve multiple independent promoters, each of which drives a single gene in the foreign DNA, have largely been unsuccessful, unless each promoter is chemically unique and the relative levels of expression of the different genes is not critical for a trait.

Tissue Culture and Regeneration

No matter how efficient the DNA transfer process, only a very small percentage of all treated cells will receive an intact foreign DNA construct at just the right time and place, and without too much cell damage to allow repair to take place. In addition, the introduced DNA must integrate into the host DNA at one or more chromosome positions where it can be expressed from its promoter. Provided all these events occur, the observable characteristic conferred by the foreign DNA should then be detectable. The problem is then to find these few cells and separate them from the millions of other cells where any or all of these events did not happen properly. To avoid having to look for these few needles in a haystack, ways are found to discard or kill selectively the unsuccessful cells. To do this, a selectable marker gene is attached to the foreign DNA in addition to the gene for the actual new trait wanted. Selectable marker genes encode proteins which make any cell which expresses them resistant to some lethal chemical such as a herbicide, an antibiotic or a toxin. Thus the 99.999 per cent of cells that were exposed to the particle gun or to *A. tumefaciens* but which failed to become transformed will die when, for example, the antibiotic is applied to the whole population. Only the survivors are likely to have the foreign DNA inserted, and can be selected for further study (see step 5 in Figure 1.3).

If kept sterile against fungal or bacterial attack and disease, each plant cell can, with careful culture conditions, become a full grown plant again. Cuttings can then be used to propagate the plant vegetatively or it can be self pollinated for seed. Each daughter plant made in this way is genetically identical to the original. Further details of this procedure are given in Appendix 2.

Once the DNA has become part of a plant chromosome it is multiplied and inherited for many generations and it is chemically indistinguishable from normal plant cell DNA. As each transgenic plant will have been regenerated from a single transformed cell, every cell in the plant will have inherited the same foreign DNA. This means that both the pollen and the ovules (and daughter seed) will carry the foreign gene(s). This potential for genes to be released into the environment explains the underlying rationale for the application of precautionary environmental risk assessment procedures (see Chapter 6).

Using Viruses, Bacteria and Fungi with Higher Plants

Sometimes it is more desirable, more feasible or more economic to genetically engineer the DNA or RNA of lower organisms such as viruses, bacteria and fungi. When these infect a plant they will then act as a transient genetic expression system within the host plant. An example of this is given in Case Study 4 with the production of vaccines from genetically engineered plant viruses. The reasons for taking this approach vary case-by-case. Often it is technically difficult to modify the genetic material of the plant itself, or it may be impossible or undesirable to do so. Sometimes, many more copies of the genetic material of the lower organism exist within each cell of the higher species than copies of its own genetic material, so that a higher yield of the desired protein can be obtained by modifying the lower organism DNA rather than the plant DNA. Similarly, sometimes the expression level of the lower species DNA (or RNA) is greater, because of the need to compete with the higher host cell, so that a higher output of the desired product can be obtained by modifying the plant virus rather than modifying the plant itself.

Gene Transfer Technology in Animals

Injecting Genes Into the Embryo

Gene transfer in animals was first achieved by the direct injection of several hundred copies of the recombinant gene into a nucleus of an early

embryo. Embryos may be obtained either by fertilisation in the laboratory, using techniques that are similar to those for human *in vitro* fertilisation (IVF), or fertilised eggs can be collected from donor animals during surgery. Hormone treatment is used to increase the number of eggs that are shed before mating or artificial insemination. The abdominal cavity is opened, the reproductive tract exposed and sterile fluid is passed through the tract in order to flush out the fertilised eggs into collecting dishes. Techniques of anaesthesia and surgery are again very similar to those used in human treatments. Whereas sheep and pig embryos have been recovered during surgery, the great cost of the technique in cattle has effectively prevented this approach. Instead, cattle embryos have been obtained from unfertilised eggs in the ovaries of slaughtered cattle. These eggs are matured in the laboratory before fertilisation and culture to the stage at which they are injected. As culture techniques improve, it is increasingly likely that embryos will be produced in the laboratory using IVF techniques, but surgical recovery of fertilised eggs will always offer the advantage of having embryos from specific, selected parents.

Injecting the DNA requires the use of microscopes because mammalian embryos at this stage are only approximately 0.1 mm in diameter and can only just be seen by the naked eye. To allow the injection, fertilised eggs are held by a small suction pipette before a very fine needle is introduced into a nucleus and the liquid containing the DNA is injected until the nucleus can be seen to swell (see Figure 1.4). A proportion of the injected eggs burst within a short period and are discarded. The surviving eggs are transferred to surrogate mothers for development to term. Although the results are variable, only approximately 10 to 20 per cent of the eggs develop to become live young, and only about 1 per cent of injected eggs develop to be born as offspring carrying an additional gene. The only way of knowing which offspring carry an additional gene is by examining DNA from each animal and this is usually obtained from a blood sample soon after birth.

In the majority of cases the added gene will be transmitted to some of the offspring of the founder. As the gene is normally only integrated into one of a pair of chromosomes it will only be transmitted to half of the offspring, and experience shows that in some cases an even smaller proportion of the offspring of the founder generation inherit the gene, apparently because the gene was not present in all of the cells from which the germ cells are derived. However, in subsequent generations the gene is usually inherited by half the offspring and it is possible to use conventional breeding to produce as many animals as required.

Gene transfer by direct injection has several limitations. It is expensive because the efficiency is low and large numbers of animals are

Photograph: Roslin Institute, reproduced by permission

Figure 1.4 *Micro-injection of DNA into a Cell Nucleus*

required, both as embryo donors and as surrogate mothers. It is believed that integration of the additional gene into a chromosome occurs because the act of injection causes breaks in chromosomes and that the repair mechanisms inadvertently include some of the injected DNA in the chromosome when repairing a break. This interpretation certainly accounts for the facts that the site of gene integration is apparently random and that in approximately 7 per cent of cases the gene has integrated within an endogenous gene, disrupting the function of that gene. It is also found that the functioning of transferred genes varies widely and it is believed that the neighbouring DNA at the site of integration influences the transgene. Finally, direct injection is only able to add a single gene. Despite these limitations direct injection has been used very widely in research and for a small number of specific applications, including the production of pharmaceutical proteins in the milk of farm animals (Case Study 8). The technique of injection has also been used to produce transgenic fish and some poultry. The technique is much more difficult to apply to poultry because access to the embryo at the appropriate stage of development is difficult. When an egg is laid, the embryo is too advanced in its development to allow injection to be carried out. Genetic engineering in poultry has therefore required the development of new techniques such as removing the fertilised eggs, injecting them and then allowing them to develop in an artificial shell. Because of these problems, genetic engineering has not, to date, been used extensively in poultry.

Modifying Cultured Embryo Cells

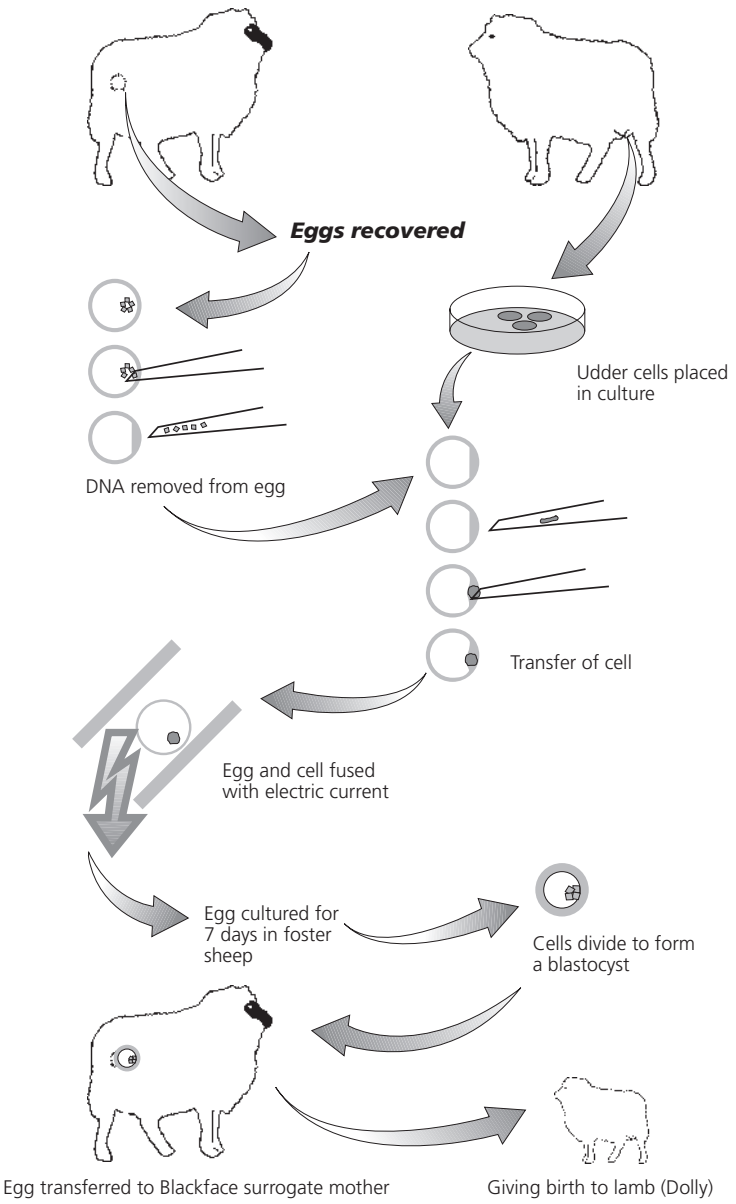
In plants, almost any individual cell can be genetically modified and then a whole new plant grown from this cell. This is not generally possible with animal cells. However, a technique has been established in mice for developing a fully grown animal from an initial cell. This technique has been used in developing many of the later examples of mouse models of human diseases referred to in Case Study 10. The technique depends upon the availability of 'embryonic stem cells' isolated from a culture of mouse embryonic cells in circumstances that allow division, but not differentiation. If such cells are introduced into another recipient embryo they sometimes retain the ability to colonise all of the tissues of the developing offspring, including the germline. The resulting offspring are derived from the cells of two embryos and are known as 'chimaeras' because some cells in the animal will have the genetic composition of the recipient and some will have the genetic composition of the donor. Offspring from some of the chimaeras, where the germline cells are from the donor, will have all their cells with the genetic composition of the donor.

While these embryonic stem cells are in culture, molecular techniques can be used to introduce precise genetic changes and in this way the change is introduced into the germline. These changes can involve deletion and modification of genes and not just addition as is possible with the micro-injection technique. It has been predicted that similar systems will probably be established in the future in other animals but, at the time of writing, embryo stem cells are not available for other species.

Transferring Nuclei Between Cells

The recently developed technique of nuclear transfer has the potential to allow the introduction of specific changes to livestock species equivalent to those possible for mice using embryo stem cells. Nuclear transfer is considered in more detail in Case Study 11 and only a brief description is given here. Nuclear transfer involves two cell types: an unfertilised egg and a donor cell (Figure 1.5). The genetic material is removed from the unfertilised egg and the genetic material of the donor cell is introduced into it, while cell development is temporarily suspended. An electric current is used to fuse donor nucleus and recipient cytoplasm and resume cell development. This has now enabled live sheep to be grown from cell cultures, achieving an end analogous to mouse stem cell technology, but by a quite different means. In each case the sheep is a clone of the animal which donated the nucleus. This was performed first with embryo cells,[6] then udder cells,[41] and then genetically modified foetal cells which produced transgenic cloned sheep.[7]

It remains to be seen how widely this can be used and in which other animals. It has now been successfully applied to cattle and mice, but only in certain cells and not with others. While the method is still at an early stage of understanding, it opens up the possibility of performing much more precise genetic modifications in farm animals at the cell culture stage, before the nuclei are used as nuclear donors. Existing methods could be used to select just the populations of cells which have incorporated the correct modification for nuclear transfer and then growth into a full sized animal. This method should enable a range of animal genetic modifications that has so far only been possible in mice. Genes could not only be added, but also removed or replaced. For example, thus far in sheep it has proved sufficient to add the gene and engineer the construct so that it is expressed in the relevant organ while the equivalent sheep gene remains active, but there are potential applications in which it would be important to switch off the animal's gene so that the human gene can perform the desired function. The new method would enable this to be done. The extension of nuclear transfer to mice also opens a wide range of medical research applications.



Source: Roslin Institute, reproduced by permission

Figure 1.5 *Nuclear Transfer Process, as Used to Produce Dolly*

Future Targets for Technical Advances

The above description of the methods used to perform genetic modification has indicated that the science is still relatively young, incomplete and subject to rapid change. Certain areas are already quite well developed, while others are at a much more rudimentary stage. This makes it especially difficult to predict the directions which future advances will take. As with all scientific knowledge, there is no finite end point to the understanding or implementation. Ideally, each new discovery brings greater understanding, but often it also reveals more complexity, with new avenues to pursue and new hypotheses to test. It is expected that overall this will lead to an increase in our basic knowledge of the physical, chemical and cell biological events which surround the uptake and integration of foreign DNA, and of how a cell regulates its own gene expression. With such knowledge, it is reasonable to expect that many procedures in use today could be made more efficient and predictable, and that some features which raise risk issues will be removed. Some of the current target areas in which technical improvements are desired – some of which are already foreseeable, others which are more long term aims – include:

- to develop more efficient methods to transfer DNA into plant and animal cells;
- to make the way this foreign DNA is then integrated into the genome of the organism more predictable and controllable than the present rather random situation;
- to enable gene deletion and replacement in farm animals using nuclear transfer methods;
- to develop ways to select transgenic material from unmodified material non-destructively;
- to design less controversial selectable marker genes for use in plants than the current methods which rely on antibiotic or herbicide resistance;
- to compile a tool-box of promoters to control how the newly introduced gene is expressed – these may be cell-, tissue-, organ-, stimulus-, time- or quantity-specific;
- to develop reliable methods and DNA construct strategies to enable multi-gene traits, as opposed to just single genes, to be transferred to crop plants or animals;
- to develop ways to prevent foreign DNA sequences being mobilised by pollen or by other germline cell types;

- to improve the efficiency of tissue culture regeneration of the more difficult species, such as cereals, grasses, legumes, woody perennials and marine and freshwater algae;
- to design more effective and broad-range pest and disease resistance genes.

In parallel, there remains much work to be done in disciplines to which genetic advances must continue to relate, such as soil science and animal physiology and embryology. The aim is for more efficient and effective end products, with no unpredicted side effects, and a consistency of performance which would merit greater confidence of society in this area of technology.

POTENTIAL APPLICATIONS OF GENETIC ENGINEERING

As a young technology in a phase of very rapid growth and development, the range of applications of genetic modification is theoretically enormous. In practice, however, there are at present some important limitations on the types of modification which can be done, and which species they can be applied to. The following summary gives four general areas where genetic engineering is currently being applied.

Food Production Applications

One of the biggest hopes from proponents of genetic engineering is that it may enable us to feed more people from the available land, in the face of the expansion of the world's population. This may be a crucial factor in helping to preserve and secure life and dignity for people, both present and future. If it is possible to develop genetically modified plants to grow on marginal land, including drought-prone, saline, eroded or desertified land, this would not only extend food production in vulnerable areas of the world, but would also help to reverse the loss of arable land through soil erosion and desertification. Table 1.1 shows the number of genetically modified foods cleared or under consideration in the UK.

In food production management, genetic engineering might make possible higher yields of milk, eggs and meat from a given number of cows, chickens or pigs. This could reduce pressures on land use, and also lower the production of methane, ammonia and other wastes from intensively reared animals. It may allow foodstuffs to be more resistant to the

Table 1.1 *Genetically Modified Foods Considered in the UK by the Advisory Committee for Novel Foods and Processes up to March 1998*[8]

Foods cleared by ACNFP	*Date cleared*
GM baker's yeast	March 1990
GM brewer's yeast	February 1994
GM soya (Glyphosate resistant)*	February 1995
Oil from GM oilseed rape (fertility restorer line, male sterile line)	February 1995
Paste from GM tomato*	February 1995
Oil from GM glufosinate-ammonium tolerant oilseed rape	May 1995
Oil from GM oilseed rape (2nd fertility restorer line)	September 1995
Oil from Glyphosate-tolerant GM oilseed rape	February 1996
Flavr Savr™ tomato (GM tomato to be eaten fresh)	February 1996
Paste from GM tomato (extension to the 1995 clearance)	February 1996
Insect-resistant GM maize-processed food products	May 1996
Ribflavin from GM ***Bacillus subtilis***	January 1997
Glufosinate-tolerant GM maize	February 1997
Insect-resistant GM maize	February 1997
Herbicide-tolerant GM maize	February 1997
Herbicide-tolerant GM cottonseed	February 1997
Herbicide-tolerant and insect-resistant GM maize	February 1997
Foods still under Consideration at March 1997	
Processed GM tomato	
Insect-resistant GM maize	
Oil from GM herbicide-tolerant oilseed rape	
Insect-resistant GM cottonseed	
GM chicory (***Radicchio rosso***)	
GM oilseed rape with high lauric acid content	

Notes: * products on sale in UK

vagaries of climate, mechanical picking and transportation. It could also be used to enhance the quality of food for the consumer and help reduce wastage of perishable foods.

Improved Yield from Crops and Animals

Of all the production characteristics to which genetic engineering might be applied, it appears that the most obvious one – that of improving the

yield of food from a crop or animal – is actually one of the most difficult. One of the features of current genetic engineering techniques is that they work best with effects which are controlled by single genes. Most of the genetic characteristics associated with yield, for example by enhanced growth, are under the control of a very large number of genes, each of which has only a very small effect on overall yield. A few genes are known which have large effects on production traits, but in animals these would need to have an advantage of at least 5 to 10 per cent in economic merit in order to be competitive with traditional breeding techniques.[9]

Early attempts at manipulating growth in animals failed due to severe welfare problems, especially with the now notorious Beltsville pig,[10] altered with human growth hormone genes. These pigs suffered deleterious consequences from the presence of this gene including gastric ulcers, arthritis, dermatitis, and renal disease. This might suggest that one of the theoretical advantages of genetic engineering – the greater selectivity in enhancing one function – may only go so far without doing harm to the overall balance of the animal's metabolism. As a result this area has not been as amenable to genetic engineering as had been hoped.

Animal production in general will probably continue to rely on conventional selective breeding, but supplemented by an *indirect* application of genetic technology, marker-assisted selection. As the genome of an animal becomes better understood, particular genes can be identified which can act as markers for various traits the breeder wishes to improve but which are not amenable to direct genetic modification.[11]

One notable exception to this pattern is in fish breeding. Trials are being carried out on salmon which have been genetically engineered to achieve a much faster growth rate.[12] Reports also suggest that transgenic sheep with improved wool growth have been produced in New Zealand.[13] Another indirect use of genetic engineering is BST (Case Study 7), in which the milk yields in cows have been boosted by repeated injections of a bovine hormone which was produced by genetically engineered bacteria. The welfare implications of this are discussed in Chapter 4.

Reduced Vulnerability of Crops to Environmental Stresses

The environment in which crops are grown has a major effect on their productivity. During their life cycle crops are subject to a range of stresses which can reduce their potential yield. These include extremes of heat and cold, drought, mineral deficiency and toxic chemicals in the soil. Case Study 2 discusses the possibility of altering crops to be resistant to these abiotic stresses. These crops would be of special importance in relation to

developing countries. Although research discoveries point to some possible applications against drought, salinity and frost, on the whole this potential has so far proved quite difficult to realise.

Increased Nutritional Qualities of Food Crops

The seeds of legumes and cereal grains provide around 70 per cent of human dietary protein requirement. Unfortunately, proteins from these sources do not provide a balanced diet because they are relatively deficient in certain amino acids required by humans. Genetic engineering could allow the modification of proteins in legumes and cereals to a form approximating more closely to the necessary balance of amino acids. This would reduce the absolute quantity of food needed and aid world food supply. Similar considerations apply to the diets of farm animals. Here, modification of the amino acid content of foods could reduce the amount of waste produced by animal husbandry systems. Given that in some countries animal wastes have become serious pollution problems, this would have certain environmental advantages. These potential developments are still at an early stage.

Increased Nutritional Qualities of Food from Animals

Milk production is an area where genetic techniques have been specially applied. In The Netherlands, genetic modification has produced a line of transgenic cattle in which the milk composition has been altered to contain the human protein lactoferrin, which is believed to make it more digestible for babies and patients on antibiotics.[14] In the UK and US, the techniques developed for producing pharmaceutical proteins in sheep's milk are also being adapted for similar enhancements of the nutritional qualities of milk in cattle.[15] The welfare and other implications of these procedures are discussed in Chapters 4 and 5.

Improved Taste, Texture or Appearance of Food

In addition to the nutritional value of food, its taste, texture and appearance all affect its usefulness and attractiveness to customers. Many of the foods which are sold in shops and supermarkets suffer losses and degradation of quality during transport and distribution. This has always been something of a problem but is especially a feature of more centralised systems in which the areas where food is produced or processed are a long way from where it is sold and consumed. This is especially the case for more exotic foods such as tropical fruits which are transported over large

distances. With fruit this often means that it is harvested before it is ripe, to reduce the chance that it will spoil before it reaches the consumer. Genetic engineering can be used to slow down this process of spoilage, so that fruit can ripen longer on the plant and then be transported to the consumer and still have a reasonable shelf-life. The Flavr Savr™ tomato in Case Study 6 has become the prime example in which a gene has been disabled, reducing greatly the rate at which the tomato softens as it ripens, so that it can be left on the vine and picked when it is more ripe and thus tastier.

Environmental Applications

Genetic engineering presents a variety of ways in which it could be possible to reduce the use of chemicals in agriculture, which could dramatically reduce the environmental impacts of current intensive farming practices. It may enable us to use a more integrated approach to crop husbandry with less reliance on chemical intervention, or use genes which confer natural resistance to pests, weeds, or viral and fungal infections. By engineering herbicide resistance into crops, there could be a faster shift from broad-spectrum chemicals to more benign ones. Genetic modification could also be used to develop crops which can produce substitutes for fuel oils, mineral oils, detergents and feedstocks for plastics. Genetically engineered micro-organisms have great potential in cleaning up various forms of pollution.

Enhanced Resistance to Weeds, Pests and Diseases, Using Less Chemicals

This is the area which has received perhaps the most attention in plant genetic engineering. Food crops suffer from a wide variety of challenges from weeds, insect or nematode worm pests, and fungal, viral, bacterial and other diseases, which cause serious problems for the farmer. The usual method to deal with these has been, for approximately 50 years, to use various chemicals which kill the organisms responsible. The resulting dependence on herbicides, insecticides and fungicides has led to environmental and health concerns, and has become a contentious issue between the agrichemical industry and those favouring more traditional organic forms of agriculture. Genetic engineering methods offer a number of alternative ways to tackle the same problems, ostensibly in a more targeted fashion, which, according to its advocates, could reduce substantially the amount of aggressive biocide chemicals being used. Initial evidence supported this claim,[16] but later results are more equivocal.

Resistance to several different herbicides has been engineered into a variety of plants. The idea is that the field can then be sprayed with herbicide which will kill the weeds but not the crop plants. The effect is produced either by increasing the tolerance of the plant to the herbicide (as has been done with the herbicide Glyphosate), or by having the plant break down the herbicide (done for sulphonyl-urea compounds). A number of crops are naturally genetically resistant to specific herbicides, like atrazine. If the genes responsible were to be isolated from resistant species and transferred into susceptible crop species, using genetic engineering, then the resistance mechanism would also be transferred. Attempts to do this by conventional plant breeding have been unsuccessful to date. The transfer of resistance should allow the more targeted use of agrichemicals and therefore reduce the amount of herbicide used.

Similar situations exist for a number of crops in relation to pest and disease resistance. Introducing genes from other plants, micro-organisms or even the pest itself can confer crop resistance and so reduce the use of pesticide sprays. For example, insect resistance has been achieved through the use of genes which produce a natural insecticide normally in a soil-dwelling bacterium called *Bacillus thuringiensis* (*Bt*). Crystals of the toxin have been used since the 1950s as an organically approved pesticide sprayed or dusted onto plants, but this type of technique is not without controversy as increased use is suspected to increase the development of B.t. toxin-resistant insects. Strategies for reducing the development of resistance have been suggested, for example including non-resistant plants in the mixture sown, and using crop rotation. However, this would depend on the integrated action of many individual farmers and therefore would be difficult to control.[17]

Work has also been carried out into obtaining resistance to insects through other means. Case Study 5 describes an example of using a genetically engineered virus to control insect pests on crop plants.

These uses of genetic engineering have been controversial. Opponents suggest they will in reality increase the use of herbicides and pesticides, and represent a cynical ploy on the part of agribusiness to increase sales at a cost to the environment. Proponents argue that plants have been engineered to be resistant to the more benign herbicides and the effect will be that of product substitution, so that the less harmful herbicides will be used rather than the more harmful ones. Nevertheless, the effect seems certain to tie genetically modified seed sales to the sales of a particular herbicide. Most of the funding of this area of genetic engineering originates with chemical companies, who are unlikely to devote considerable resources to the development of seeds which have no need of their products.

Reduced Dependence on Fertilisers and Other Agrichemicals

In addition to herbicides and pesticides, fertilisers are the major chemical additive used in agriculture, adding essential elements to improve the production and viability of crops. Nitrogen is one of the most important elements controlling crop productivity. Over 60 million metric tonnes of nitrogenous fertilisers are applied annually world wide. This is projected to increase to 160 million metric tonnes in the foreseeable future. However, a number of economically important crop plants, such as Soya beans, clover and other legumes, have evolved a symbiotic relationship with soil bacteria of the group *Rhizobium* which allows them to extract nitrogen directly from the air. This means that they do not require the application of nitrogenous fertilisers. Theoretically, this nitrogen fixing facility could be extended to plants currently without it, using genetic engineering techniques.

In practice, the introduction of the ability to fix nitrogen into plants is difficult and very complex, involving at least 15 different genes. The aim is to introduce either the ability to fix nitrogen directly or the ability to form a symbiotic relationship with *Rhizobium*, but either result is some way off yet. If this is successful, it could have a major impact upon world food supply, but it is also possible that it may not turn out to be quite the 'philosopher's stone' that is hoped.

Another application to chemical processes in agriculture involves genetically engineering microbial products to increase their efficiency at modifying the acidity and other properties of silage and hay. These microbes can dramatically reduce the losses in storage after harvesting which are caused by contaminating organisms.

Production of Novel Substances in Crop Plants

Increasingly, genetic engineering is being applied to both plants and animals for novel uses other than food – this is covered in several of the case studies. Oilseed is mainly used at present for margarine and various other food oils, but genetic modification (Case Study 3) could extend the range of such crops to include the production of fatty acids for detergents, substitute fuels and petrochemicals. Many of these applications are at an early stage, but they offer the prospect of significant environmental benefits – in cutting our dependence on non-renewable fossil fuels, and reducing net greenhouse gas emissions, since the carbon dioxide emitted by burning a biofuel is only that which the plants had originally absorbed from the atmosphere. Case Study 4 indicates the considerable potential

use of crops for the production of vaccines and other pharmaceuticals by means of genetically modified plant viruses, with obvious medical benefits. If these various applications eventually become viable on a large scale, they will raise important questions of land use next century, because significant quantities of agricultural land would need to be set aside for energy crops, raw materials and medicines, instead of food. Although in the present UK context of over-production leading to surplus land this would appear to be a welcome development, that situation may not always apply and very different circumstances exist in many other countries of the world. Moreover, as discussed in Chapter 9, some of the genetic modification of oilseeds may have significant adverse economic effects on developing countries.

Use of Bacteria in Pollution Control

Bacteria can now be genetically engineered with designer enzymes and whole metabolic pathways capable of degrading a wide range of highly toxic man-made environmental pollutants (such as chlorinated hydrocarbons, pesticides, waste from munitions factories) to less dangerous or shorter-lived chemicals, or to mop up toxic heavy metals; processes known collectively as bioremediation.

Medical and Veterinary Applications using Animals

One of the developments in genetic engineering often regarded as most exciting is the range of novel medical benefits derived from animals. This has become the most important area of animal genetic engineering.

Pharmaceutical Proteins in Animals

Perhaps the most advanced application to date has been the production of therapeutic proteins in the milk of sheep, goats and cattle by introducing genes of human origin into the animal. The leading example is given in Case Study 8. There appear to be excellent prospects for a wide range of proteins to be produced by such methods, some of which are difficult to obtain by other means.

Xenotransplantation

A second area is the controversial modification of pigs or other mammals to enable their hearts, kidneys and other organs to be used for transplant

into humans, seeking to overcome the rapid and catastrophic rejection of tissue from a foreign species that otherwise occurs. This is being developed in response to the growth in demand for transplant organs which in many countries has far outstripped the practicable supply. Case Study 9 describes the main UK work in pigs. There are uncertainties about ethical acceptance by the public, and the future of this work is also unclear in the face of concerns about the risk that it could lead to animal diseases being transferred to humans.

Animal Models for Human Diseases

The most widespread of all animal applications, however, has become the use of genetically modified mice as models for human disease and to test potential therapies. Partly because of the existence of embryo stem cell technology in mice, the application of genetic engineering to mice has been greater than to other animal species. Many different transgenic mice have been produced which have been genetically manipulated to produce various different diseases which are found in humans. The most famous of these mice is the oncomouse which has been genetically engineered to have a predisposition to develop cancer, described in Case Study 10.

Animal Disease Resistance

It may be possible to use genetic engineering to assist in combating some animal diseases, and to improve the resistance of farm animals to some diseases. This is a relatively undeveloped area, partly because the genetic traits associated with animal disease resistance, while of potentially great value, are often dependent on several genes. Possible applications include cattle with increased resistance to mastitis or sheep which have an insecticide against blow flies in their wool. A postulated application of animal cloning could be to produce a number of identical cloned animals which could then be compared in different environments in order to evaluate their susceptibility to diseases such as mastitis in cattle.[18]

Economic and Social Benefits

The governments of industrialised nations have long seen biotechnology as a rich potential source of wealth and job creation, akin to the information technology revolution. Biotechnology already employs many tens of thousands of people and may generate substantial profits for companies and their shareholders. This in turn should create wealth in the wider

society when they are spent or reinvested. Much of this potential is still a long way off, however. High capital investment in research and extensive regulations for product testing and validation mean long lead times from the much heralded breakthrough in science to a company beginning to see profits from the product. It remains to be seen to what extent the economic benefits reach the wider society and if they are fairly distributed. There could also be economic benefits for more marginal agricultural areas of the world if genetic modification enables products to be grown on poor yielding land. On the other hand, if it becomes possible to grow, for example, palm oil substitutes on temperate farms in richer nations, hard-pressed tropical economies could suffer.

The field of genetics also presents many fascinating research problems for the scientific expertise and intellectual development of a country. The understanding which genetic research is bringing to the way living organisms work is a major addition to human knowledge, notwithstanding the ethical challenges this inevitably also brings, and which this book seeks to explore.