
# Transgenic animals

UNIT 11

*European Initiative for Biotechnology Education*

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***The European Initiative for Biotechnology Education (EIBE) seeks to promote skills, enhance understanding and facilitate informed public debate through improved biotechnology education in schools and colleges throughout the European Union (EU).***

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*European Initiative for Biotechnology Education*

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# About this unit

These materials have been devised by practising teachers and educationalists from several European countries, brought together with financial support and encouragement from DGXII of the European Commission, under the auspices of EIBE, the European Initiative for Biotechnology Education.

The EIBE materials have been extensively tested in workshops involving teachers from across Europe.

The views expressed in this Unit and the activities suggested herein are those of the authors and not of the European Commission.

# Unit materials

This unit is unusual since it is divided into various sections, and thus offers opportunities for flexibility in approach, particularly with respect to timing.

The ***Introduction*** gives background information on how transgenic animals are produced and covers the main uses.

The three sections - ***A mouse against cancer, Sumosalmon*** and ***Transgenic sheep***, are examples of the main applications of transgenesis in animals, and they employ different strategies. Any one of these sections can be used as a stand alone, or in any combination (any two or all three).

***A mouse against cancer*** illustrates the use of transgenic animals as disease models and is a role-play situation involving researchers and management in an imaginary company. It not only deals with the production of a transgenic mouse that carries a gene that promotes the development of a brain tumour in humans, but also includes economic and ethical considerations.

***Sumosalmon*** deals with growth promotion. This is also a role-play exercise involving the setting up of a fish-farm in a seaside village for the production of giant transgenic salmon. It takes the form of a public debate.

***Transgenic sheep*** is a paper exercise, giving information about emphysema and its genetics, and how it could be alleviated by the development of transgenic sheep that produce α-1 proteinase inhibitor in their milk. It also considers this human enzyme in the treatment of Cystic Fibrosis and cloning. There are questions to be answered, which include ethical considerations.

Since societal issues are emphasised in parallel with scientific concepts, the materials would be appropriate to both science and non-science teachers and students.

INTRODUCTION

# Background information

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Over the centuries, animals with new combinations of genes have been produced using conventional breeding methods by means of careful selection of particular animals. The number of new gene combinations that can be achieved in this way are, however, limited since genes can only be shuffled about between members of the same or very closely related species.

Transgenesis is a radically new technology for altering the characteristics of animals by directly changing the genetic material. Since DNA contains a universal genetic code for living organisms, it can, in principle, be transferred between completely unrelated organisms to produce organisms with particular, useful characteristics that would not otherwise be available.

Many different genes and their action have now been characterised. This knowledge opens up the possibility of looking for methods of changing genes in ways that are useful; for example to cure diseases or to introduce desirable genes into an animal for a variety of reasons.

This unit is concerned only with the genetic modification of animals (transgenic animals) - other EIBE units which are also concerned with genetic modification are *Unit 9* and *10* (Transgenic plants I and II) and *Unit 14* (Novel Foods).

## Risks and benefits

Transgenic technology in animals is still experimental. With time and experience, it could become commercially viable. At this experimental stage it is possible to see both potentials benefits and to predict possible risks in the new techniques that are becoming possible.

### *Benefits*

***Specificity***
*The characteristic required can be chosen with much greater accuracy and additional unwanted traits can be kept to a minimum.*

***Speed***
*A desired characteristic could be established in one generation, many generations are usually needed for selective breeding.*

***Flexibility***
*New characteristics (cross species) become a possibility*

***Economy***
*Characteristics may be introduced for animals that will need fewer feed supplements and medical treatments*

### *Risks*

***Animal health***
*Insertion of a transgene may upset the expression of the genome (and the consequent functioning of the animal).*

***Virus transfer***
*This is of particular concern in animals bred as tissue donors for xenotranplantation.*

***Dissemination***
*Normal reproduction may result in a transgene being released to the wild population.*

## Famous sheep

** TRANSGENIC *(see pages 7-8)* * CLONED *(see page 8)*

*Scientists at The Roslin Institute in Scotland have played a key role in the development of this technology and the following sheep, bred at Roslin, have had international fame:*

### Tracey (born ) ** TRANSGENIC

Tracey produces human α-1 proteinase inhibitor in her milk, she was produced using the technique of pronuclear injection. This procedure involved the introduction of 200 - 300 copies of the transgene into recently fertilised eggs, only 2-3% of treated eggs gave rise to transgenic offspring and only a few of those expressed the added gene at useful levels (see *Microinjection, page 7*).

### Megan and Morag (1995) * CLONED

Megan and Morag were cloned from embryo cells by nuclear transfer. Their genes were not modified in any way, but they were the result of a successful attempt to show that it was possible to derive live lambs from embryo cells that had been cultured for several months in the laboratory. (When animals can be derived from cells in culture it is possible to carry out much more specific genetic modifications - see *Embryonic stem cell transfer, page 8*).

### Dolly (1996) * CLONED

The birth of Dolly showed that nuclear transfer could work even with cells from an adult, she also was not genetically modified but was derived from cells that had been taken from the udder of a six year old sheep. In April 1998, it was announced that Dolly had been mated and had produced a healthy lamb, Bonnie.

### Polly (1997) ** TRANSGENIC * CLONED

Polly is the first transgenic lamb to be produced by nuclear transfer (*see page 8*). She was produced from foetal fibroblast cells that were modified by the addition of the human gene coding for blood clotting factor IX (linked to a promotor gene causing expression in the sheep mammary gland) together with a marker gene (neomycin resistance).

## Constructing a transgene

Although the genetic code is essentially the same for all organisms, the fine details of gene control differ. A gene from a bacterium will, for example, not often work correctly if it is introduced unmodified into an animal cell. The genetic engineer first of all constructs a transgene - containing the gene of interest plus some extra DNA that correctly controls the function of the gene in the new animal. This transgene has then to be inserted into the new animal.

Many genes are only expressed in particular tissues and are controlled by a special segment of DNA next to the gene called a promoter sequence. When constructing a transgene, scientists generally substitute the donor's promoter sequence with one that is specially designed to ensure that the gene will function in the correct tissues of the recipient animal. This is crucial when, for example, the gene needs to be expressed in the milk of a mammal.

As well as the promoter DNA sequence the transgene requires a poly A sequence so that it will function correctly (see Fig.1).

## Inserting the transgene

There are several methods of inserting a transgene, the following are examples of techniques currently used.

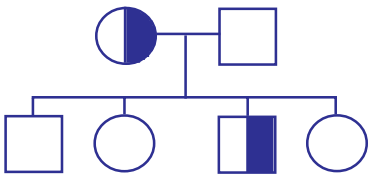
### 1. Microinjection

In this method eggs are harvested from super-ovulated animals and fertilised in vitro. A micro-tube is used to hold the fertilised egg in place and an extremely fine needle used to inject a tiny amount of a solution, containing many copies of the foreign DNA (transgene), into the male pronucleus. These eggs are then introduced into the oviducts of surrogate females.

***Figure 1. A transgene***


This is the main method currently used to produce genetically modified animals and involves the physical injection of 200-300 copies of the foreign gene into recently fertilised eggs and their subsequent implantation into surrogate mothers. Only a small percentage of the animals born are transgenic (i.e. they carry the added gene from one generation to the next) and only a proportion of these express the added gene at a high level. Genes can only be added (not deleted) by this method.

Such founder animals can be mated with non-transgenic animals to produce animals which are heterozygous for the gene.



Heterozygotes can in turn be mated to obtain animals which are homozygous for the foreign gene.

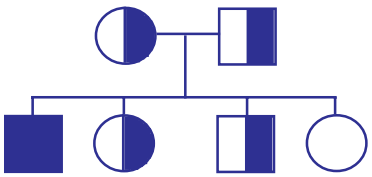
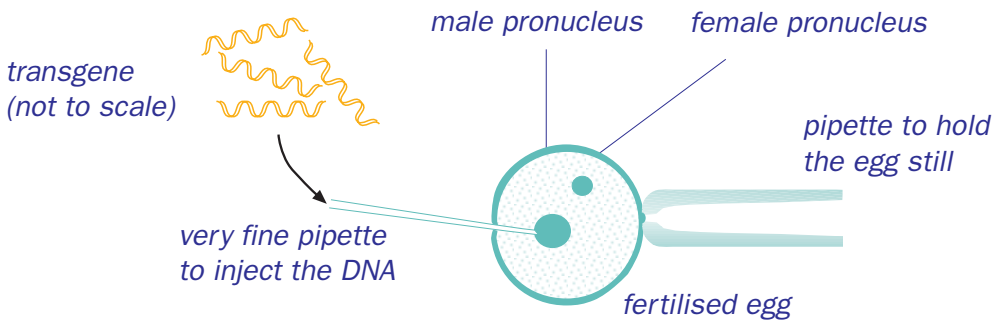


***Figure 2. Microinjection***



### 2. Use of retroviral vectors

These viruses can be used to carry the gene sequence of interest into embryonic cells. However, as in microinjection, the gene is still inserted randomly into the genome. Since the DNA locates at different sites in different cells the offspring are often genetic mosaics and outbreeding is necessary to obtain pure lines.

### 3. Embryonic stem cell transfer

This method, less random than methods 1 and 2, is used when it is important to target gene sequences to specific sites in the genome.

When cells are in culture it is possible, using appropriate vectors, to carry out specific genetic modifications; such as removing or substituting a chosen gene, or even changing an individual base in the genetic code. Embryonic stem cells, modified in this way, can be injected into the blastocyst stage of a developing embryo and the resulting foetus will be a chimera (usually in all organs, including the gonads). Further breeding can select for the modified trait. This method has been used in mice but as yet has not been successful in cattle, sheep or pigs.

All the methods described above have been used to produce transgenic mice - transgenic livestock have been obtained so far only by the use of microinjection or nuclear transfer. The modification of the technology derived from work on mice for farm animals has not been straightforward. The efficiency of transgenesis is low and it is both more time-consuming and expensive when working with large animals. With time and experience, there is no doubt that it will become an important new area in biotechnology.

## Nuclear transfer *(cloning)*

The nucleus of an unfertilised egg can be removed and replaced with the nucleus of a donor cell (which contains the complete genome). An electric current is used to fuse the cells and trigger the egg to begin developing. The 'reconstructed eggs' are then implanted in surrogate mothers.

Donor cells are normally obtained by culturing embryonic cells; new techniques are being developed to use 'quiescent' cells from mature tissues.

The birth of Polly (*see page 6*) has shown that somatic cells can be cultured, subjected to genetic manipulation in vitro, and then produce viable animals by means of nuclear transfer.

Nuclear transfer has the advantage that the sex of the transgenic animal is predetermined.

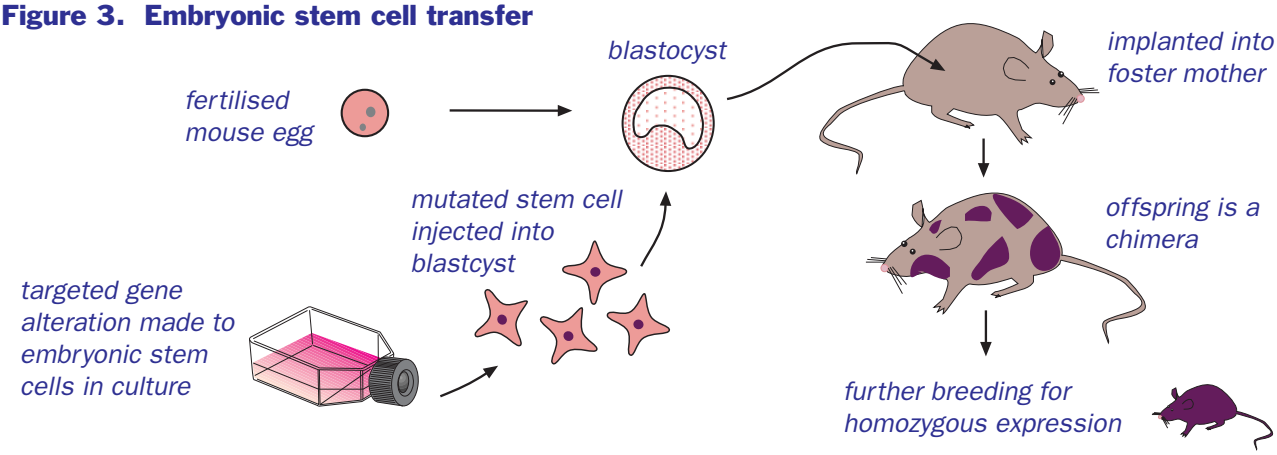
## Applications of Transgenesis

- **Disease models**
  Human mutant genes may be inserted into mice, causing them to suffer from human diseases, treatments can then be devised without experimenting on humans (see section - *A mouse against cancer*).

- **Improving livestock**
  Livestock can be produced that are faster-growing, leaner, convert food more efficiently and resist diseases (see section - *Sumosalmon*).

- **Molecular pharming**
  Livestock are used to produce medicines and nutraceuticals. Transgenic sheep, goats and cattle are being used as 'bioreactors' to produce important human proteins in milk (see section - *Transgenic sheep*).

**Figure 3. Embryonic stem cell transfer**



DISEASE MODELS

# A mouse against cancer

## a case study for discussion

### Setting the scene

The Pharma Company, AnyGene in Manchester used to be one of the most successful companies in the field of gene technology applied to medicine. They specialised in research into and treatment of hereditary diseases. Their highly successful research centred on methods of transforming bacteria by inserting the genes coding for proteins needed for the treatment of various hereditary diseases, such as special forms of diabetes. For about eight years the company was very successful, selling the medicines produced by these transgenic bacteria. During this time other companies in various parts of the world were also successful in this field of research, creating considerable competition and causing a dramatic drop in Pharma's profits.

In an attempt to overcome this problem, Pharma's management first of all fired fifty employees, a quarter of the workforce. Secondly, it was clear that in order to keep the investors happy and to get the company out of the red and making good profits, new innovations were essential. If they were not successful over the next three years in finding means of obtaining more information about hereditary diseases and in producing medicines to prevent them then the company would have to close. This in turn would of course mean that all the employees - scientists, workers, secretaries etc. would lose their jobs. They were aware that with the present poor economic situation world-wide the chances of finding new jobs would be small.

The people most concerned were the researchers. During a 'crisis meeting' they decided that they would change the focus of their research, bacteria were not the best choice for discovering the biochemical and physiological mechanisms and the genetic basis of hereditary diseases in humans. What they really needed were human beings to work on - since this was impossible the next best option was a model organism for humans. They decided to work with mice and to focus their research work on cancer since they had recently detected, analysed and cloned a gene for a certain form of brain cancer (called *brac 1* in their scientific jargon). This form of brain cancer was very aggressive and particularly painful and it affected all ages - many fatal cases had occurred in teenagers - at present there is no cure.

The aim of the researchers was to 'construct' a transgenic mouse that would carry the gene *brac 1* and thus would be susceptible to this special form of brain tumour. Such transgenic mice could then be used as a disease model for investigations into the development of the brain tumour and to try out medicines to prevent its growth.

After their initial meeting the head of the research team discussed with the management the decisions that had been reached and went on to outline research plans for the next two years. The managing director was pleased with the plans but some other members of the management team objected to them. They drew attention to a particular rule in the company policy stating that any genetic modification of mammals had to be fully discussed and decided upon by an ethics commission. It was agreed to set up an ethics commission consisting of researchers, management and experts on ethics.

# Background information

## Animals as disease models

Among products of genetic modification techniques transgenic animals are the most spectacular. By transferring foreign DNA into animal cells new genes can be inserted or functioning genes can be switched off. In some cases such new genes can be transmitted to the next generation. This technique can be used, for example, to create cows that produce proteins in their milk that are important in medicine (see section on transgenic sheep). In addition, transgenic mice have been found to be very valuable in investigations into gene function and for the analysis of different hereditary diseases.

During the development of a mammal the genome is fixed by the time it is fertile. Egg and sperm cells each carry a single (haploid) copy of the hereditary information. Only in special cases e.g. by infection by a virus, is additional genetic material integrated into the genome - such material does not however affect the germ cells, so that it is not passed on to the offspring.

Several factors such as radiation, chemical mutagenesis and errors in DNA-replication can lead to the loss or destruction of genetic information. If such mutations occur in a germ cell then they can become part of the genome. Most mutations are recessive, happen by chance and are disadvantageous to the organism. Only on the odd rare occasion is the mutation of benefit to the organism. It is thought that such mutations are the cause of genetic variability on which natural selection works bringing about evolutionary processes.

From the viewpoint of a geneticist, particular mutations are valuable because they can be used to indicate the presence of a particular gene in the genome. Since however such mutations only occur by chance geneticists working with mammals have to wait until they take place.

To overcome this problem scientists worked out techniques to insert a particular gene into a mammalian genome. Using gene technology techniques they succeeded in cloning particular mammalian genes. This enabled their structure and sequence to be analysed. Over the last ten years techniques have been developed whereby genes can be modified in a test tube and then reinserted into mammals so that the effect of the new gene on the development and biological characteristics of the transgenic mammal could be studied. New techniques also allowed particular genes to be switched off so that they were no longer a functioning part of the genome. The precondition for the insertion or elimination of genes is that it is possible to transfer into the cell recombinant DNA that integrates itself firmly into the DNA of the mammal.

## Microinjection - *one way to insert a foreign gene into a mouse*

The most direct way of integrating a new gene into a cell is to inject a piece of DNA into the nucleus in the hope that it will become integrated into the genome. This sounds unlikely but it does actually work. This technique is called **microinjection**(*see also page 7*).

Microinjection is the most frequently used method of inserting a foreign gene into the genome of a mouse in order to develop a model for investigations into hereditary diseases.

### Preparation of the DNA

The DNA to be integrated has first to be cleaned - this is done using standard molecular biology techniques. The DNA is then modified so that it contains regulation elements (e.g. promoter, stop-codon etc.) of a gene and the protein coding sequences of

another gene. The prepared DNA is then inserted into a vector and propagated in bacteria. The DNA pieces can be separated from the vector DNA using restriction enzymes.

**Preparation of the recipient**
Mice embryos are obtained by treating *'virgin'* mice with hormones so that their cycles are synchronised and super ovulation occurs, producing a higher number of egg cells than normal. After fertilisation, early embryos are collected and analysed under a special microscope.

**Microinjection** *(see also page 7)*
About 8 to 12 hours after fertilisation the pronuclei can be detected. Each egg cell contains two pronuclei containing the genetic information either of the mother or the father. Only into one of these pre-nuclei is the modified DNA injected. With each microinjection 50 to 500 copies of the modified DNA-fragments are inserted into the pre-nucleus.

**Development**
Not all of the embryos survive the mechanical damage caused by inserting the needle. On average 60 - 80% of the embryos survive the microinjection process. The embryo is then transferred to the oviduct of a falsely pregnant female mouse. Female mice are made falsely pregnant by mating them with sterilised male mice. As a result these female mice enter the hormone cycle of pregnancy without carrying any embryos.

The implanted embryos develop normally in the surrogate mouse, after birth they are left for about 3 weeks with their surrogate mother. They are then analysed to find out if the injected DNA has integrated into their genome. If it has integrated into the genome it will have replicated together with the rest of the genome during each cell division of the embryos growth so that it should be present in every cell. If the injected DNA has not integrated it will not be detectable.

When the mice are about 3 or 4 weeks old their DNA is taken from a piece of tail tissue. Using the Polymerase Chain Reaction technique (PCR, see *EIBE Unit 2*), it is multiplied and analysed to find out if the foreign DNA is present in the mouse's genome. Generally it is found that 15-30% of the mice are transgenic. The new genes are often very active so that some of the transgenic mice developed really exhibit new attributes.

## Transgenic mice and cancer

What triggers cells to abandon their normal behaviour and to divide out of control to produce a tumour? Why do cancer cells move to other parts and organs of the body to produce tumours there as well? Is the change from a normal cell to a cancerous cell a consequence of genetic change, external factors, a disturbance of the immune system, or is cancer only a result of growing old?

We now know that cancer has many different triggers. It can be caused by smoking, by nutritional factors, by radiation and by chemicals. These are all factors external to the body. In addition, it has been found that for several kinds of cancer like breast, colon, brain and skin cancer, genes, called 'oncogenes' are involved. In the main however, it seems that the outbreak of cancer is the result of a combination of environmental factors and genetic disposition.

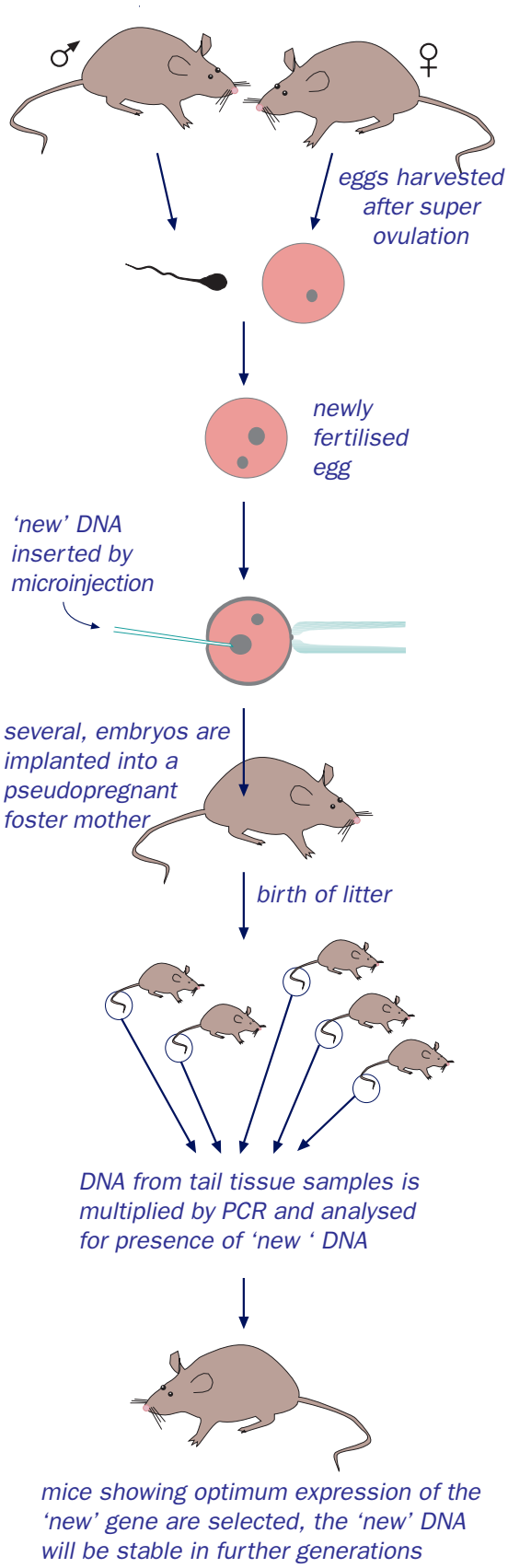
Through investigations with transgenic mice that have been modified using a particular oncogene and thus developed a certain type of cancer, questions concerning the relationship between oncogenes and cancer development could be answered. Theoretically such animals can also be used for research into cancer treatment and cancer prevention.

In the laboratory of Philip Leder in Harvard (USA) the transgenic mouse model for the investigation of breast cancer was developed. Two discoveries were of great importance. The first was the identification of a regulating element in the mouse mammary tumour virus (MMTV) that is active specifically in cells of the mammary gland. Secondly, he and his group identified and cloned oncogenes. The oncogenes *myc* and *ras* were analysed to find out if they lead to breast cancer in mice transformed with these genes.

In transgenic mice examples were found where a single oncogene caused cancer in the tissue of the mammary gland. One example was the *neu*-oncogene; this codes for a protein that serves as a receptor for a growth hormone. In all mice transformed with the MMTV and the x*neu*-oncogene, cancer broke out, usually a short time after puberty.

Other oncogenes also caused the development of breast cancer in transgenic mice. Depending on the particular oncogene the tumours look different under the microscope. This indicates that each oncogene contributes differently to the development of cancer.

**Figure 5. The steps in the production of transgenic mice**



STUDENT ACTIVITY 1

# A mouse against cancer

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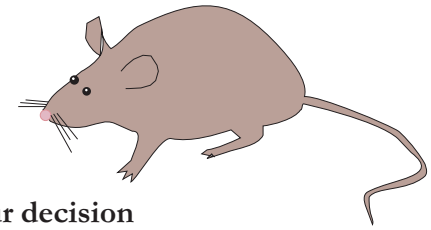

1. **Identify the question**
   First of all - is there a dilemma? A dilemma occurs when there is no 'right' course of action in a particular situation but several options, none of which is wholly acceptable. Ethical dilemmas revolve around trying to find the best solution when no particular solution is completely good.

2. **Identify the issue**
   What is the general topic in this case?

3. **List the facts**
   What are the facts in this case?
   Be careful - stick to the text. Do not make assumptions of your own into facts! Do not jump to conclusions!

4. **State possible decisions**
   What are some possible solutions to the problem? List as many as you can.

5. **Make up your mind**
   Which of the following groups do you wish to belong to?

   - ***The researchers who are in favour of using transgenic mice as disease models.***
   - ***The ethics experts.***

   There should now be two groups in the class. Each group should from now on work separately.

6. **Study the information**
   You are given the following information to help you to come to an informed decision :
   Animals as disease models (*page 10*).
   Microinjection - one way of putting a foreign gene into a mouse (*pages 7,10,12*)
   Transgenic mice and cancer (*page 11*).

7. **Select your decision**
   Go back to point 4 and select the decision that you find reasonable for the point of view of your group, keeping in mind all the information that you have received. Having decided you have to find out what principles you are abiding by and what principles you are breaking. State these principles and write them down. Then state your decision in the form "I think that the commission should decide that ....., because ..... ."

8. **Commit yourself to one principle**
   Find out which principle is the main one affecting your decision.

9. **Find support from experts**
   Which experts would you back up on this point?

10. **Consider alternatives**
    Under what circumstances would you change your mind about what to do?

11. **Classroom discussion**
    Each group should outline their answers to questions 6 and 10. Discuss the two results and work out why they are different.

FOOD

# Sumosalmon

## History of domestication

The mammoth has now disappeared. Nature has always selected between living things. Humans interfered in this process about 10 000 BC with the taming and breeding of particular wild animals - this was the beginning of domestication. With domestication, the phenotypic variability of domesticated species rose as numbers increased. This resulted from a greater variability with the increased numbers and better survival and reproduction than in the wild due to human protection against predators.

The 'hind-end' of cattle is a feature that has been recognised for over a century. At the beginning it was considered rather abnormal. Today an animal with a good hind-end is in demand for the quality of the meat. Besides their muscle hypertrophy, there are associated drawbacks such as: difficulties in calving requiring Caesareans, poor viability of calves (rachitis, functional heart abnormality, even myopathy) and poor fertility. Without human aid such mutants would have been eliminated by natural selection.

Wild animal populations present a more uniform phenotype than domestic animal populations.
The modern notion of animal breeds appeared in England in the 18$^{th}$ century together with the industrial revolution. At that time, the foundations of intensive agriculture and breeding were laid in order to meet the consumption needs that were required by developing urban and industrial communities. Rational selection of sires based on a limited number of preferred characteristics was first made and breeds achieved from the artificial selection of the most useful members of domestic animal species. 'Natural' breeding is a myth.

Artificial selection brought about an increase in production rates. At the beginning of the century, a dairy cow provided 2 000 to 3 000 litres of milk a year. Today Holstein cows provide 6 000 litres on average, and up to 8 000-10 000 for the best ones. A century ago, a hen laid about 70 eggs a year whereas today the best races lay up to 250 eggs per year.

Crossbreeding between members of the same species aims to improve useful features. There are however barriers to cross-breeding between species which are not always impenetrable, e.g. when a mare is crossed with a donkey the resulting mule is sterile. These impenetrable barriers have now been overcome with the technique of transgenesis whereby genes can be transferred within and between microbes, animal and plant species.

## Growth hormone gene transfer

On 8 September 1981 the first successful transgenesis was achieved by Wagner and his team from the University of Ohio, jointly with the Jackson Laboratory at Bar Harbor, Maine. Wagner transplanted a rabbit's β-globin gene into a mouse embryo.

In 1982 Brinster and Palmiter successfully injected into the eggs of a mouse the gene which controls the synthesis of growth hormone. Some of the resulting mice grew very large. In the first experiments, the transgene came from a rat; later, a human gene was preferred.

As a result of such experiments the U.S. Secretary of Agriculture supported future research in the hope of producing larger animals. It was hoped that 'giant' cows as huge as elephants and able to produce 15 000 litres of milk a year' could be produced.

Such cows could also mass-produce substances medically useful to humans such as growth hormone. These useful proteins could be produced in the milk of these cows if the gene for the protein was linked to a control (promoter) sequence that is found next to a gene synthesising a milk protein e.g. casein. The U.S. Secretary of Agriculture also hoped to replace classic fermentation vessels using *E.Coli* bacteria used in the pharmaceutical industry by 'natural' fermentation vessels (bioreactors), namely giant transgenic cows. Pharmaceutical substances would thus be synthesised in the milk and then separated from it.

These projects were seriously considered and the U.S. Secretary of Agriculture financed Brinster and Palmiter so that they could carry out feasibility studies. A biotechnology firm Biosym was set up in 1983 in the USA with the aim of making giant domestic animals. In 1985 the results however proved to be disappointing because although the transgenes were well integrated into the chromosomes they did not function properly.

Subsequent experiments in other countries where the growth hormone gene has been transferred to cattle, pigs and sheep have also not been very successful. For example, pigs which were not giant but possessed less fat, were found to show symptoms of arthritis and stomach ulcers which were often fatal. Also, due to hormone disequilibrium, females had no oestrus so that they were sterile.

On the other hand, transgenic fish which carried a transgene which synthesised growth hormone were produced.

# Information for the teacher

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## Presentation

This role play is a decision making exercise. Students are confronted with an imaginable but fictional situation. They have to decide whether or not to approve a giant trans-genic 'Sumo' salmon farm being set up in a seaside village. They participate in a public debate proposed by the mayor.

## Aim

By taking part in the role play, students learn to :

- understand that making decisions can be complex when there are important social issues involved - economic, ethical and ecological;
- understand the principles underlying genetic engineering;
- express and defend, or criticise, the viewpoints adopted by the individuals they are representing;
- distinguish between descriptive (describing facts) and normative discourse (evaluating facts) in a discussion.

The role play exercise is important in the development of decision making, value clarification, and problem solving in the social context. Morry Van Met's book: *The Effective Use of Role Play. A Handbook for Teachers and Trainers*, Kogan Page Ltd, London, 1983, is an excellent source of information for becoming more familiar with role play.

A questionnaire assesses student attitudes towards animal transgenesis in general, and on Sumosalmon in particular. The questionnaire can be used in a variety of ways. It can be used as a pre-post test to assess the development of students' attitudes; as a pre-test to promote the students' involvement; as a post-test, or simply as topics for further debate (*see Appendix, page 30*).

Thirteen individuals are described, and any extra students can participate as observers. The characters are for or against the proposal to produce Sumosalmon. The number of individuals allows participation in the role play of 15 to 20 students. It is preferable if all students are involved. It is possible to carry out the role play with less than 13 individuals, but there should be a balance between those for and those against. If there are more students than available roles, these can be allocated to groups of students, and after discussion one student of the group can be selected to play the role. The cards describing the individuals' and the observers' tasks are distributed at random.

## Suggested sequence

After transgenesis has been introduced (possibly with a presentation of the history of domestication and the background of growth hormone gene transfer), students fill in the questionnaire (*see appendix, page 29*) on attitudes (15 minutes).

After the subject and interest of the role-play have been presented, students express and justify their opinions on the setting up of a Sumosalmon farm (15 minutes).

Once the roles have been handed out, students make up a list of questions that they wish to ask, and express their arguments, naturally from their role's viewpoint (15 minutes). The students know which individual characters are going to be involved in the role play. The teacher distributes labels on which are written the names and the job/role specification of the participants. The observers should organise themselves in order to gather together their observations.

Role-play is under way (30 to 45 minutes). The teacher plays the role of the mayor. He/she introduces the role-play and is responsible for the timing and encouraging the students in the exchange of questions, arguments and information. Then the teacher (the mayor) asks the group to come to an agreement on the proposal to be submitted at the next local council meeting.

At the end of the role play, each individual participant expresses his/her opinion on the proposal (15 minutes) and specifies under what circumstances he/she would change their mind.

After the role play, students fill in the questionnaire on attitudes again. The method and individual feelings (not in role play) are discussed (30 minutes). The decision-making process is analysed with the assistance of the observers (15 minutes).

STUDENT ACTIVITY 2

# Sumosalmon

## The wild salmon's life

From December to January, salmon stay in the cold and rapid waters of streams with gravel beds on which the females rub their bellies to release their eggs. Eggs are then sprayed by the male sperm in order to be fertilised. Hatching takes place in February-March. When they are two years old, young salmon swim downstream to reach the marine environment. When adults, they swim back up the native stream to spawn. While in the sea, salmon may stay very far away from their native river. Salmon from Norway, Scotland and England can be found in the same sea, whereas they will take different routes to go back to their native river to spawn.

## Research on Sumosalmon production

In 1994, Canadian geneticists from the Vancouver Fisheries and Ocean Department (British Columbia) jointly with two researchers from America and Singapore created a line of giant transgenic salmon. At one year old they are on average eleven times as big as normal salmon of the same age. One even reached a growth rate thirty seven times normal. They are the famous Sumosalmon.

Experiments on fish had given disappointing results until this time, since they were based on transferring a mammalian gene. For these successful experiments, researchers used genetic material from salmon.

The growth hormone transgene was injected into 3 000 fertilised eggs, whose development had been inhibited just after fertilisation, by micro-injection into the blastodisc. After one year, the transgene was effective on 6.2% of the surviving alevins (young salmon) which had an impressive growth rate. This genetic modification also accelerated the sexual maturation of these fish which were capable of breeding and transmitting their growth capabilities to their offspring. The technique however is not yet fully controlled - some embryos do not survive the modification since as the transgene is placed randomly in the recipient genome it can upset the expression of other parts of the genome or the expression of the transgene itself.

## Setting the scene

In a seaside village close to a fishing harbour, a fish farmer, Yann Le Goff, is planning to breed genetically modified salmon which grow more rapidly and become giant, called Sumosalmon (from the name of Japanese wrestlers). The local population is very concerned about this project. A group including fishermen, consumers, conservationists, and traditional fish farmers formed a committee to fight against this project. However, Yann Le Goff rallied support from the owner of the canning factory and a part of the local council. The mayor decided to organise a public debate with specialists in the field.

ROLES

# Sumosalmon

## Roles

| | |
|---|---|
| **Yann Le Goff** | *fish farmer* |
| **Captain McCook** | *owner of the canning factory* |
| **Briac Prigent** | *traditional fish farmer* |
| **Yvon Le Bihan** | *master fisherman* |
| **Nathalie Delalande** | *'with it' media studies student* |
| **François Le Fur** | *gastronome* |
| **Marie Queffelec** | *fishmonger* |
| **Jean Le Naour** | *leader of an environmental association* |
| **Alex Garnier** | *researcher* |
| **Jérémie** | *Surf Rider Foundation* |
| **Stéphanie Jennet** | *young mother* |
| **Jules Fontaine** | *Mayor* |
| **Félix Adambounou** | *PhD student in biotechnology* |

## Observers

# Yann Le Goff fish farmer

You are about 30 years old. You have just taken over the running of the family fish farm. You must pay inheritance shares to your brother and sister.

The price of salmon is decreasing due to international overproduction. To meet your liabilities, you want to raise your farm's productivity without increasing your production costs. So you are planning to produce large salmon more rapidly. During your training before setting up, you heard about the latest research concerning Sumosalmon production. You are negotiating a contract with the owner of the canning factory.

# Captain McCook owner of the canning factory

You are about 50 years old. Your grandfather and your father owned the canning factory. You have developed a traditional firm of renown. You produce various sorts of canned fish such as sardine, tuna fish and mackerel, as well as smoked salmon. Your suppliers are the local fishermen and fish farmers.

To keep up with social change - increasing number of women working outside the home, increased leisure time, and decrease in time devoted to preparing meals - you plan to set up a processing industry to produce ready prepared fish dishes. To achieve this you require a steady supply of large quantities of salmon. You hope that Yann Le Goff will succeed with his project of breeding giant Sumosalmon so that he will become your main supplier. Sumosalmon will provide a larger number of steaks of similar size and their flesh seems to be good raw material for low-calorie ready-made dishes. Given the demand for low-calorie food for reasons of both health and body image, you expect a sound added value and increased sales.

However, you are worried about consumers' possible rejection of the produce if you have to mention genetic modification on the packaging.

# Briac Prigent traditional fish farmer

You are 55 years old. You breed salmon in the traditional manner. Intensive fishing practices have impoverished natural resources so that the amount of caught wild salmon dropped about twenty years ago, leading to a rise in prices. Like some others, you decided then to set up a very costly salmon farm. However, this has led to over production with resulting falls in prices. Naturally you are worried about Yann Le Goff's Sumosalmon project.

Still, you hope to be able to maintain your market share based on consumers' choice for 'naturally bred' fish.

# Yvon Le Bihan master fisherman

You are 50 years old. You have always worked on a boat. A ship's boy at the age of 14, you managed to become a master fisherman. You catch a wide range of different fish. Salmon farming competes more and more with your activity. However you think that consumers will be able to tell the difference and appreciate the flavour of wild fish caught in the sea.

You have heard that the salmon farm 'cages' do not always contain 'domestic' fish. Norwegian fjords are said to abound with lines of 'domestic' fish that have escaped from immersed cages into the open sea during storms. Between 5 and 30% of the fish caught in the sea come from fish farms. What if these Sumosalmon escaped accidentally! Would they ruin the whole ecosystem by eating large numbers of fish? You are naturally very worried.

# Nathalie Delalande *'with it'* media studies student

You are 20 years old. You are studying communication sciences. You would like to get a job in the advertising sector. You are keen on everything new, that is the reason why you are attending this debate. In principle you are enthusiastic about the project of breeding Sumosalmon. You believe that it is necessary to move with the times and to innovate.

You pay attention to your diet, you like ready-made meals and low calorie food since you want to keep slim.

# François Le Fur gastronome

You are a 50 year old lawyer. You are the president of a fine gourmet association and have written a book on traditional gastronomy. Your fishmonger assures you that the fish he sells is not bred in fish farms. You think that breeding Sumosalmon is a scandal : "A human gene may have been implanted in salmon, and they practically want to make us eat human flesh". Producing transgenic animals is unnatural. These unnatural animals may carry unknown diseases. You believe that it is necessary to be watchful, especially after the mad cow disease issue.

# Marie Queffelec fishmonger

You are 40 years old. You are the owner of the fish shop situated in the centre of the village. You are suffering more and more from super- and hyper-market competition. You sell up-market fish. You worry a lot about Yann Le Goff's Sumosalmon project. You fear above all that consumers will panic and stop buying fish without making any distinction. You do not even know whether the fact that Sumosalmon have been genetically modified will be mentioned on the labels.

# Jean Le Naour leader of an environmental association

You are 30 years old. You are a village councillor on the opposition bench and you often disagree with the mayor. You are very well informed about the research carried out on the Sumosalmon, and you totally disapprove of Yann Le Goff's project. According to you, Norwegian fjords abound with lines of 'domestic' fish that have escaped from the immersed cages in the open sea during storms. Between 5 and 30% of the fish caught in the sea come from fish farms.

You raise many questions : If these transgenic salmon escaped accidentally, should we be afraid of ecosystem disequilibrium? Can Sumosalmon not contribute then to a reduction in biodiversity? Would it be possible for the transgene to be transmitted to wild salmon? What could be the consequences? How can Sumosalmon be reliably confined to prevent them from escaping?

# Alex Garnier researcher

You are 45 years old. You carry out research in fish physiology. The mayor asked you to participate in the public debate as an expert. Naturally you are very familiar with the research work on the Sumosalmon. According to you, transgenesis is not highly efficient -only 6.2% of the surviving alevins express the transgene- and with the drop in 'classic' salmon prices, you believe that the economic validity of Sumosalmon production has to be checked.

Norwegian fjords abound with lines of 'domestic' fish that have escaped from the immersed cages into the open sea during storms. Between 5 and 30% of the fish caught in the sea would come from fish farms. You know that research, urged by the European Union, on ecological hazard due to Sumosalmon' accidental escaping has just started. Should we be afraid of ecosystem disequilibrium? Will there be a further reduction in biodiversity?

In order to prevent the transgene from being transmitted to wild salmon, your research team is working on methods to sterilise the fish.

# Jérémie Surf Rider Foundation

You are 20 years old. You are studying biology at the university. You are a surf rider and a member of the Surf Rider Foundation which is concerned about ecological issues on the seaside such as building construction, water pollution etc. You are against genetic manipulation in principle. As to Sumosalmon, you feel particularly concerned about the ecological hazards if they happened to escape. From what you have heard, Norwegian fjords abound with lines of 'domestic' fish that have escaped from the immersed cages into the open sea during storms. Between 5 and 30% of the fish caught in the sea come from fish farms.

You raise many questions : If these transgenic salmon escaped accidentally, should we be afraid of ecosystem disequilibrium? Can Sumosalmon not contribute then to reduce biodiversity? What could be the consequences?

# Stéphanie Jennet young mother

You are 25 years old. You have studied geography at university. You are the mother of Louise, who is just one year old. You find it important to feed her with organically-grown products. You use alternative health care for her. Being opposed to any unnatural treatment, of course you are against the production of transgenic animals, notably Sumosalmon. You are particularly afraid that these products might surreptitiously penetrate the market without you knowing about it, unidentified, unlabelled, as it often happens for food irradiated to give it a longer shelf life, or beef from herds with BSE.

# Jules Fontaine Mayor

You are 55 years old. You won the latest elections by a narrow margin. You support Yann Le Goff's Sumosalmon project, but you worry about your electors' reactions; this is the reason why you are organising this debate.

# Félix Adambounou PhD student in biotechnology

You are African. You are 28 years old and a PhD student under the supervision of Alex Garnier. You have a research scholarship looking into the production of African transgenic fish, i.e. the production of fish which could express a growth hormone gene construct. This biotechnology is considered of great importance to your country because of concerns with malnutrition. The production of giant fish could increase the availability of proteins. Convinced of the importance of transgenesis, you are afraid that its high cost prevents its application in your country, especially if transgenic animals are patented.

# Observers

You will analyse the debate. and must therefore be able to distinguish between two types of discourse :

- **descriptive discourse** - describing facts, e.g.
  *a) the size of salmon can be modified by genetic engineering;*
- **normative discourse** - evaluating a fact or an action, e.g.
  *b) it is bad to modify the size of salmon by genetic engineering ; humans are not entitled to modify nature;* or
  *c) humans are entitled to modify the size of salmon by genetic engineering because it will result in increased fish farm production.*

Gather together the different participants' speeches.
Note points for and against in a table.
Make a selection of the major arguments and sum them up.
Make a list of descriptive and normative arguments.

# Transgenic Sheep

## The problem

Emphysema is a lung disease in which the lung tissue is damaged and this causes severe breathing difficulties in some people in their thirties and forties. It mainly occurs in people who are susceptible to the disease for genetic reasons and who are exposed to airborne irritants like cigarette smoke, chemicals etc. Those who smoke account for about 2% of patients with emphysema.

Breathed-in air contains many living things, for example spores and bacteria, which must be broken down before they can cause any harm. Our lungs contain large numbers of neutrophils: white blood cells that secrete enzymes which break down foreign proteins. One of the important enzymes that the neutrophils secrete is elastase, which digests any foreign proteins or irritants which enter the lungs via the air-stream. The more irritants there are in the air, the more elastase is produced.

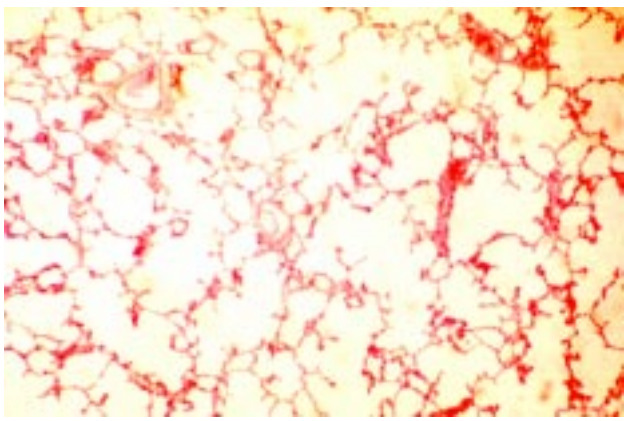
The walls of the alveoli in the lungs contain elastin which maintains the elasticity of the lungs, and this can also be broken down by elastase. To prevent this from happening there is secreted into the blood serum an enzyme called $\alpha_1$-proteinase inhibitor (previously called $\alpha_1$-antitrypsin) or αPI, which can bind to the elastase, thus inactivating it and protecting the lung tissue from damage. There is a careful balance set up between the two enzymes. If αPI is missing then the elastase is not controlled and it attacks the elastic fibres in the lungs. The result is rather like taking a pair of scissors to a fishing net - the holes (alveoli) become larger. The photomicrographs, see Figure 6, are at the same magnification.

Since large spaces appear in the lungs, the surface area through which gas exchange can take place is greatly reduced, the lungs become less efficient, and they also become more prone to damage by pollutants.

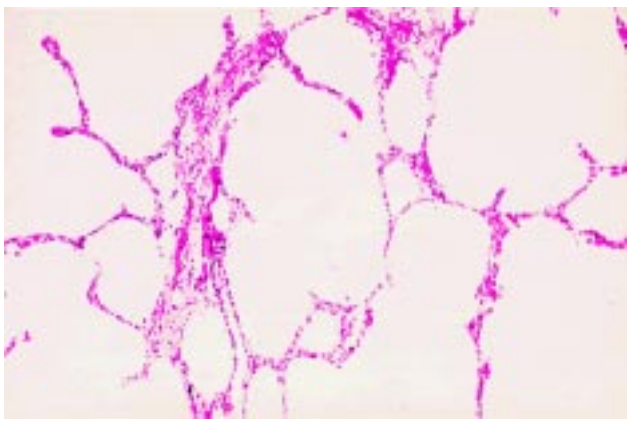
### Genetics of $\alpha_1$-proteinase inhibitor

The production of αPI is, like all proteins, under genetic control. The genes for αPI production are located on chromosome 14. Let us call the gene for αPI production, M. Most people possess two copies of this gene: they are thus MM and are relatively

**Figure 6. Lung tissue**



*Normal lung tissue*



*Lung tissue from a person suffering from emphysema*

safe from lung disease unless lung irritation is excessive as in heavy cigarette smoking. About 5-10% of the population carry a mutant M gene - let us call it Z, so they have the genetic make-up MZ (about 9% of Irish people carry at least one Z gene, whereas it is rare to find it among Italians and American Indians). MZ people do not produce as much αPI as they should. About one in two thousand people carry both copies of the mutant gene: they are ZZ and don't produce any αPI at all.

In summary (this is a simplification of the real situation since another gene S is involved):

| | |
|---|---|
| MM | normal production of $\alpha_1$-proteinase inhibitor |
| MZ | reduced production of $\alpha_1$-proteinase inhibitor and so more susceptible to emphysema. |
| ZZ | no production of $\alpha_1$-proteinase inhibitor and so very susceptible to emphysema. |

From research it has been found that :

- ZZ smokers develop emphysema about 9 years earlier than ZZ non-smokers.
- ZZ individuals are generally more susceptible than others to a whole range of respiratory irritants found in industry.
- MZ individuals who are exposed to smoke or other irritants are more likely to develop respiratory complications than their MM colleagues.
- Low levels of αPI have been found in a larger number of TB patients in Greece than can be explained by chance.

## Questions

1. If you were young would you like to know if you were MM, MZ or ZZ? No matter which way you answered, explain your reasoning.
2. If you found that you were an MZ individual, would this prevent you from taking up smoking, or, if started, make you give it up? Again, no matter which way you answered, explain your reasoning.

Many diseases have environmental aspects as well as genetic. The following people work in environments which can lead to lung disease:

- Suberosis in wine makers, from mouldy cork.
- Cheese worker's lung, from mouldy cheese.
- Sequoiosis in mill workers, from mouldy redwood dust.
- Maple bark stripper's disease in maple syrup collectors, from mouldy maple bark.
- Baggassosis in sugar cane workers, from mouldy sugar cane.
- Farmer's lung, from working with mouldy hay.
- Wheat weevil disease in granary workers and bakers, from infested wheat flour.
- Bird breeder's lung, from pigeon and parakeet droppings.

## Question

3. Should workers in the above industries be screened to find out if they are MM, MZ or ZZ?

Many people could obviously benefit if a cure could be found for this problem of lack of αPI.

# The solution

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MZ and ZZ individuals could be identified at birth, or even earlier, using genetic markers. This chronic condition could be prevented, or at least minimised, in individuals who do not produce their own αPI or at a low level, in two ways.

- Gene therapy, of which there are two possibilities:
  a) somatic gene therapy, where the normal gene is put into lung tissue, and this brings about production of αPI;
  b) germline gene therapy, where eggs that are missing the normal gene have it inserted into the nucleus of the egg. Such genes would then be inherited by subsequent generations.

- Drug treatment, administering αPI as an aerosol (in the same way as using inhalers with asthma sufferers). High doses of the inhibitor would be required - about 4 grams per week (200 g/year).

## Gene therapy

Research is still problematical regarding gene therapy, but progress in this area is being made in the case of cystic fibrosis (see *EIBE Unit 4*). At present method (a) above, i.e. administration via nebulizer, appears to be the most promising.

## Pharmaceuticals

Normally αPI is extracted from human blood plasma, but the amounts available are small and the cost excessive. Many people could benefit from this treatment if αPI could be produced in large enough quantities at a price that could be afforded. Pharmaceutical Proteins Ltd. of Scotland decided to try to find if they could get sheep to produce this enzyme in their milk.

### Pontential advantages

- Sheep are mammals and so would produce αPI which is more like that found in humans than if made by bacteria in a bioreactor.
- Sheep are cheaper to use than cows, since they mature more quickly.
- Milk is very easily collected.
- The enzyme would be formed only in the milk, not in the rest of the animal, so the sheep would remain fit and healthy.
- Large quantities of enzyme could be produced, since a flock of such sheep could be bred.
- The enzyme would be easily purified from the milk.
- The enzyme could be produced cheaply.

### The method

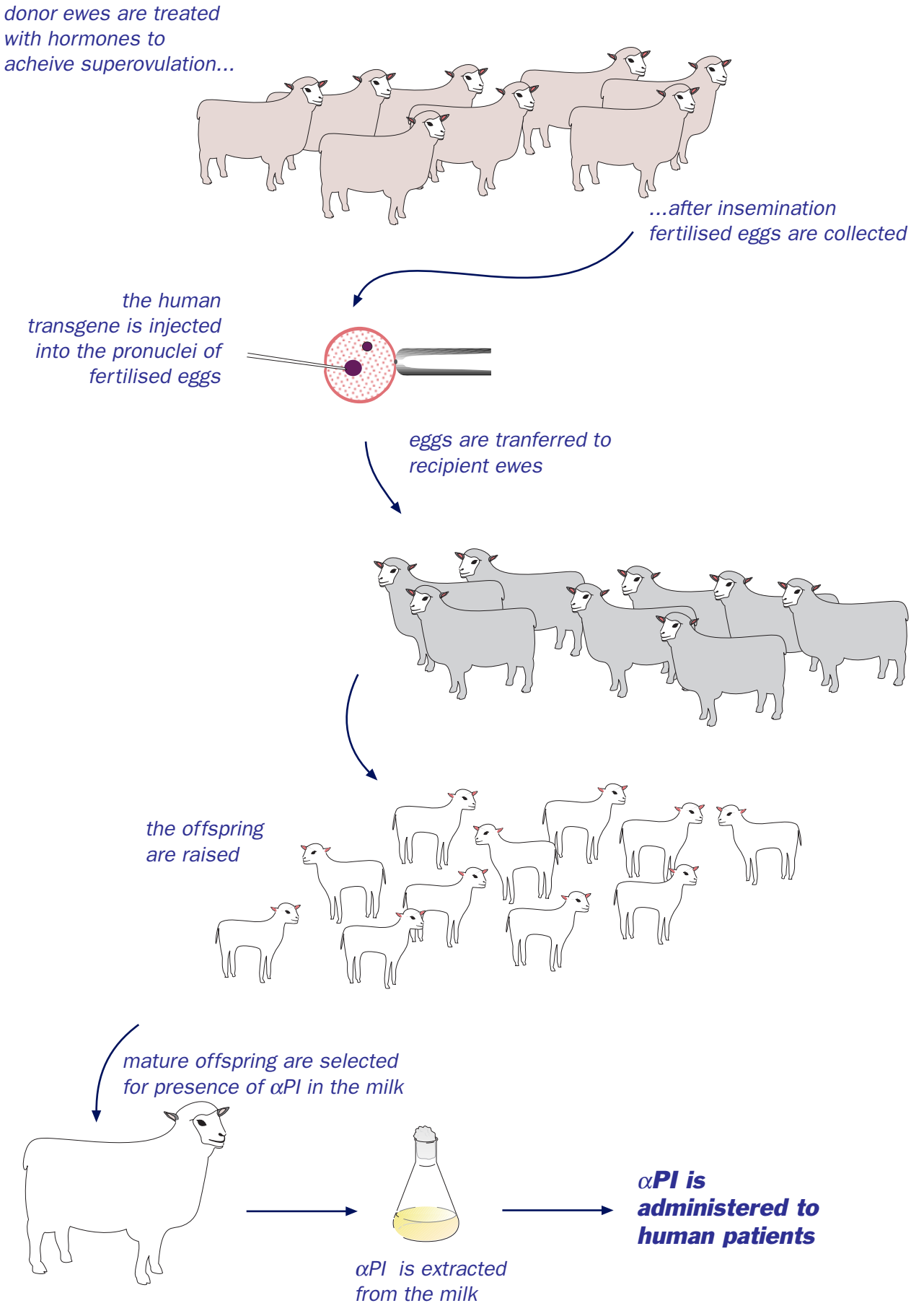
The steps necessary for the production of a human protein in sheeps milk are outlined in Figure 7 (page 26).

The crucial stage in this process is inserting the human gene into sheep, thus the term transgenic. This technique is called genetic manipulation, genetic engineering or genetic modification, but they all mean basically the same thing. By using transgenic animals, in theory it would be possible to produce any human protein that is required for therapeutic purposes.

Once the milk is obtained it is skimmed to remove the fat. The proteins in it are then precipitated and separated using column chromatography when the αPI fraction is separated off. It is possible using these techniques to recover about 30% of the aPI in the milk.

Such a method of production using 'furry bioreactors' is very cost effective. One of the ewes, Tracy, produces milk with more than 30 grams of αPI in every litre of milk. In her first lactation she produced one and a half kilograms of αPI. There is a high concentration of the enzyme in the medium which in turn is very cheap - one litre of milk costs only a couple of pounds sterling to produce. The sheep are perfectly fit and healthy and do not seem to have

**Figure 7. The steps in the production of $\alpha_1$-proteinase inhibitor in sheep milk**



have had any ill effects from producing αPI in their milk.

So, using the new techniques of gene technology, a very complex enzyme which behaves in identical fashion to the human enzyme, is capable of being produced in a large enough quantity at a low enough price, so that potential sufferers of emphysema can be protected, and existing sufferers offered some relief.

Once the enzyme is produced this is not the end of the story. It has to receive approval from the regulatory agencies after extensive clinical trails. This can take a number of years. Hopefully it will not be too long before αPI find its way on to the market.

The Managing Director of Pharmaceutical Proteins Ltd, Dr. Ronald James, continually receives letters which indicate the need for this product. One such letter from a Mr. Smith (that is really his name) was as follows :

*In the issue of the Daily Telegraph on Thursday, April 5th, there appeared an article dealing with your efforts to produce antitrypsin in viable quantities for the relief of emphysema, and as far as I know, I am in the earlier stages of this condition. When sitting still, I can breathe normally, but walking causes me to breathe hard and any activity more strenuous than walking sets me fighting for breath. Apart from that, my health is very good indeed for a man of my age. It has occurred to me that in the course of your research, you may require to test your products on human beings. If this need arises, I should be only too happy to offer my services in this respect. I am well aware that there may be an element of risk associated with clinical trials but, at my age and in my condition, I am quite happy to chance the possible ill effects in the hope that good will ultimately reward your efforts and perhaps be of benefit to me too.*

### Question

4. If you were identified as ZZ, would you offer your services for clinical trials? No matter what your answer, explain.

## Treatment of Cystic Fibrosis

αPI is also under clinical trials at present to find out how effective it is in the treatment of cystic fibrosis (see *EIBE Unit 4*).

The lungs of CF sufferers are prone to infection by the bacterium *Pseudomonas*. The body reacts to such infections by increasing the production of neutrophils (white blood cells), which digest the bacteria. To aid this process they produce large amounts of elastase; the bacteria in turn try to prevent the elastase from attacking them by producing an alginate coat. The result of this 'battle' is that the lungs and airways contain large amounts of elastase which attacks the epithelial cells in the lungs and also breaks down the αPI.

We have already seen that there is normally an excess of αPI over free elastase but in the case of CF sufferers it is the other way around; an excess of elastase and reduced levels of αPI. The balance needs to be restored by supplying αPI to the patient by nebuliser.

If the clinical trials are successful then regular supplies of relatively cheap αPI would be invaluable in helping CF sufferers.

### Questions

5. Are there any aspects of this technique of producing human proteins using transgenic farm animals that disturb you?
6. What alternatives to pharming (as it is now often called) do you think there are?

## Cloning

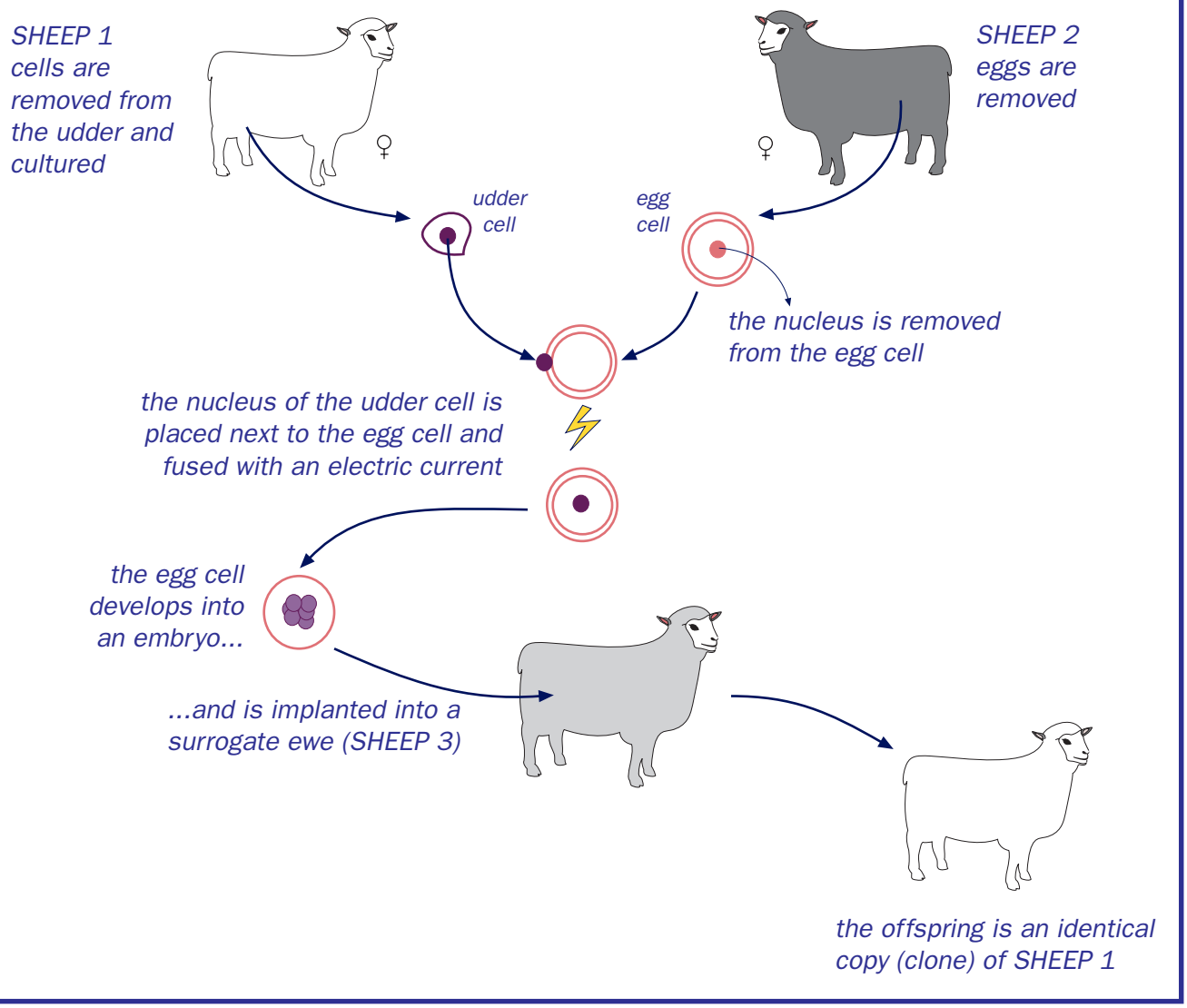
Early in 1997 the news broke that a sheep called Dolly had been born, she was the result of an experiment at the Roslin Institute where the work on the production of transgenic sheep had taken place. They had succeeded in producing a clone from a somatic cell of an adult sheep. All around the world the media blazed with fantastic headlines like 'The cloning of Dolly questions the status of human life and procreation' (The Irish Times, Feb.26, 1997). Very few articles actually explained why the researchers had cloned a sheep.

When a human gene is inserted into a sheep egg the probability that it will become incorporated into the genome and be expressed in the way required e.g. as a protein in the milk, is very small. It is a chancy business with a low probability of success so that many attempts have to be made before success is achieved. Once a sheep has been found that produces large quantities of the required human protein in its milk, cloning techniques could be used to produce a whole flock of cloned sheep that could produce all the αPI (or any other human protein) required.

### Question

7. In your opinion was it important that the scientists were able to clone sheep?
8. Do you think that in the near future people will be cloned? Should this happen.

**Figure 8. The cloning of Dolly**



THE FUTURE

# Whatever next?

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The last few years have seen rapid advances in the techniques of transferring genes and the associated technology of animal cloning. In the future transgenic animals may be developed for a wide range of purposes and an even wider range of ethical issues will have to be considered.

Some of the areas in which transgenic animals may well be important in future developments are:

**Medical research**

- in many areas, this was the first, and is currently the main, use of trangenic animals.

**Livestock improvement**

- resistance to infection
- growth efficiency

**Pharming**

- biologically active human proteins
- diagnostics human immunoglobulins (monoclonal antibodies)
- nutraceuticals

**Xenotransplantation**

- as organ donors.

## Some potential areas of concern:

Transgenic animals will be developed by commercial organisations and maintained for commercial reasons, will there be any national or international breeding banks set up to maintain valuable (but non commercial) transgenic animals.

There are strict regulations and procedures that apply to the release of transgenic organisms. In what way will the environmental impact assessments and population dynamics of transgenic animals be monitored and co-ordinated.

How will the need for commercial confidentiality be balanced against the need for openess concerning safety and animal welfare.

## World Wide Web pages

Home pages are given below to sites that contain material on transgenic animals or cloning at the time of writing. Many also have links to further sites of interest.

**Biotechnologyand Biological Sciences Research Council:**
www.bbsrc.ac.uk
**CAB international:**
www.cabi.org
**CSIRO:**
www.its.csiro.au
**Human Genetics Advisory Commission:**
www.dti.gov.uk/hgac
**Medical Research Council:**
www.mrc.ac.uk
**Nature:**
www.nature.com
**New Scientist:**
www.newscientist.com
**Roslin Institute:**
www.ri.bbsrc.ac.uk

APPENDIX

# Questionnaire

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Human milk is vital for new-born babies at risk and for premature babies. It is needed in maternity wards. Research work is under way into using cows to produce 'humanised' milk. It would be obtained from animals with one or several genes integrated into their genome, enabling them to synthesise human milk proteins.

1. *Do you agree that researchers should seek to develop such transgenic cows?* 1. YES ❑ NO ❑

Third world countries concerned with malnutrition and high infant mortality rates could plan to produce 'humanised' milk.

2. *Do you think that it would be beneficial to third world countries to produce 'humanised' milk?* 2. YES ❑ NO ❑

3. *Do you think that it would be beneficial to industrialised countries to produce 'humanised' milk?* 3. YES ❑ NO ❑

4. *Would you tgive such 'humanised' milk to babies?* 4. YES ❑ NO ❑

Transgenesis has not been well mastered yet and it is costly.

5. *Do you think that transgenesis will be within third world countries' reach?* 5. YES ❑ NO ❑

Research work is under way in order to obtain transgenic cows which could produce better quality milk for making cheese.

6. *Do you agree that researchers should seek to develop such transgenic cows?* 6. YES ❑ NO ❑

In your opinion, who would be interested in this research?

7. *Farmers who make cheese?* 7. YES ❑ NO ❑

8. *Dairy industries?* 8. YES ❑ NO ❑

9. *Cheese consumers?* 9. YES ❑ NO ❑

10. *Researchers?* 10. YES ❑ NO ❑

Research work is under way to obtain transgenic animals resistant to certain diseases.

11. *Do you agree that researchers should seek to develop cattle resistant to certain diseases?* 11. YES ❑ NO ❑

12. *Do you agree that researchers should seek to develop pigs resistant to certain diseases?* 12. YES ❑ NO ❑

13. *Do you agree that researchers should seek to develop fish resistant to certain diseases?* 13. YES ❑ NO ❑

Some cancers are inherited. Transgenic mice which have received a gene causing cancer have been developed so that cancer mechanisms can be studied, and anti-cancer treatments improved.

14. *Do you agree that researchers should seek to obtain such mice?* 14. YES ❑ NO ❑

Transgenic ewes have been developed that secrete human proteins of pharmaceutical benefit in their milk, for example a protein to treat people suffering from emphysema.

15. *Do you agree that researchers should seek to obtain such ewes?* 15. YES ❑ NO ❑

Research work is under way to obtain giant transgenic fish for farming.

16. *Do you agree that researchers should seek to obtain such fish?* 16. YES ❑ NO ❑

17. *Would you eat this fish?* 17. YES ❑ NO ❑

Some African countries are thinking of making up for food protein deficiency with fish.

18. *Do you think that these countries are likely to develop the breeding of giant transgenic fish?* 18. YES ❑ NO ❑

For comparative results please contact Dr. Laurence Simonneaux: laurence.simonneaux@educagri.fr